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(54) Title: HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE

(57) Abstract

The present invention is directed to imparting stress resistance to plants. This can be achieved by applying a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart stress resistance to plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the elicitor can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart stress resistance to plants or plants grown from the plant seeds.

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HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE

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FIELD OF THE INVENTION

The present invention relates to imparting stress resistance to plants with a hypersensitive response elicitor.

BACKGROUND OF THE INVENTION

Under both natural and agricultural conditions, plants are exposed to various forms of environmental stress. Stress is mainly measured with respect to growth (i.e. biomass accumulation) or with respect to the primary assimilation processes (i.e. carbon dioxide and mineral intake). Soil water deficits, suboptimal and supraoptimal temperatures, salinity, and poor aeration of soils may each cause some growth restrictions during the growing season, so that the yield of plants at the end of the season expresses only a small fraction of their genetic potential. Indeed, it is estimated that in the United States the yield of field-grown crops is only 22% of genetic potential. The same physicochemical factors can become extreme in some habitats, such as deserts or marshes, and only specially adapted vegetation can complete its life cycle in the unusually hostile conditions. In less extreme environments, individual plants can become acclimated to changes in water potential, temperature, salinity, and oxygen deficiency so that their fitness for those environments improves. Some species are better able to adapt than others, and various anatomical, structural, and biochemical mechanisms account for acclimation.

Under natural and agriculture conditions, plants must constantly endure stress. Some environmental factors can become stressful in a very short period of time (e.g., high or low temperature) or may take long periods of time to stress plants (e.g., soil water content or mineral nutrients). Generally, environmental stress effecting plants can be in the form of climate related stress, air pollution stress,

PCT/US99/26039

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chemical stress, and nutritional stress. Examples of climate related stress include drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light. Air pollution stress can be in the form of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain. Chemical stress can result from application of insecticides, fungicides, herbicides, and heavy metals. Nutritional stress can be caused by fertilizers, micronutrients, and macronutrients.

For most plants, water is essential for growth. Some plants are able to preserve some water in the soil for later use, while others complete their life cycles during a wet season before the onset of any drought. Other plants are able to aggressively consume water to save themselves while causing water deprivation for other plants in that location. Plants lacking any of these capabilities are severely hampered by the absence of water.

Chilling injury occurs in sensitive species at temperatures that are too low for normal growth but not sufficiently low to form ice. Such injury typically occurs in species of tropical or subtropical origin. When chilling occurs, discoloration or lesions appear on leaves giving them a water-soaked appearance. If roots are chilled, the plants may wilt. On the other hand, freezing temperatures and the accompanying formation of ice crystals in plants can be lethal if ice crystals extend into protoplasts or remain for long periods.

Stress is also caused by the other temperature extremes with few plants being able to survive high temperatures. When higher plant cells or tissues are dehydrated or are not growing, they can survive higher temperatures than cells which are hydrated, vegetative, and growing. Tissues which are actively growing can rarely survive at temperatures above 45°C.

High salt concentrations are another form of environmental stress which can afflict plants. In natural conditions, such high concentrations of salt are found close to seashores and estuaries. Farther inland, natural salt may seep from geological deposits adjoining agricultural areas. In addition, salt can accumulate in irrigation water when pure water is evaporated or transpired from soil. About 1 all irrigated farmland is effected by high salt concentrations. High salt content not

only injures plants but degrades soil structure by decreasing porosity and water permeability.

Air pollution in the form of ozone, carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, and hydrocarbons can very adversely effect plant growth by creating smog and environmental warming.

The present invention is directed to overcoming various forms of environmental stress and imparting resistance in plants to such stress.

SUMMARY OF THE INVENTION

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The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart stress resistance. Alternatively, stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

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Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air polllution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients). Applicants have found that use of hypersensitive response elicitors in accordance with the present invention impart resistance to plants against such forms of environmental stress.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one

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embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart stress resistance. Alternatively, the stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

The hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include Erwinia, Pseudomonas, and Xanthamonas species (e.g., the following bacteria: Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora, Pseudomonas syringae, Pseudomonas solancearum, Xanthomonas campestris, and mixtures thereof). In addition to hypersensitive response elicitors from these Gram negative bacteria, it is possible to use elicitors from Gram positive bacteria. One example is Clavibacter michiganensis subsp. sepedonicus.

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

| | Gly 65 | Ala | Ser | Ser | Lys | Gly 70 | Leu | Gly | Met | Ser | Asn 75 | Gln | Leu | Gly | Gln | Ser 80 |
|-----|------------|------------|-------------|------------|--------------|------------|------------|------------|------------|------------|------------|------------|--------------|------------|------------|------------|
| | Phe | Gly | Asn | Gly | Ala 85 | Gln | Gly | Ala | Ser | Asn 90 | Leu | Leu | Ser | Val | Pro 95 | Lys |
| 5 | Ser | Gly | Gly | Asp 100 | Ala | Leu | Ser | Lys | Met 105 | Phe | Asp | Lys | Ala | Leu 110 | Asp | Asp |
| | Leu | Leu | Gly 115 | His | Asp | Thr | Val | Thr 120 | Lys | Leu | Thr | Asn | Gln 125 | Ser | Asn | Gln |
| 10 | Leu | Ala 130 | Asn | Ser | Met | Leu | Asn 135 | Ala | Ser | Gln | Met | Thr 140 | Gln | Gly | Asn | Met |
| | Asn 145 | Ala | Phe | Gly | Ser | Gly 150 | Val | Asn | Asn | Ala | Leu 155 | Ser | Ser | Ile | Leu | Gly 160 |
| | Asn | Gly | Leu | | Gln 165 | Ser | Met | Ser | Gly | Phe 170 | Ser - | Gln | Pro | Ser | Leu 175 | Gly |
| 15 | Ala | Gly | Gly | Leu 180 | Gln | Gly | Leu | Ser | Gly 185 | Ala | Gly | Ala | Phe | Asn 190 | Gln | Leu |
| | Gly | Asn | Ala 195 | Ile | Gly | Met | Gly | Val 200 | Gly | Gln | Asn | Ala | Ala 205 | Leu | Ser | Ala |
| 20 | Leu | Ser 210 | Asn | Val | Ser | Thr | His 215 | Val | Asp | Gly | Asn | Asn 220 | Arg | His | Phe | Val |
| | Asp 225 | | Glu | Asp | Arg | Gly 230 | | Ala | Lys | Glu | Ile 235 | Gly | Gln | Phe | Met | Asp 240 |
| | Gln | Tyr | Pro | Glu | Ile 245 | | Gly | Lys | Pro | Glu 250 | Tyr | Gln | Lys | Asp | Gly 255 | Trp |
| 25 | Ser | Ser | Pro | Lys 260 | | Asp | Asp | Lys | Ser 265 | Trp | Ala | Lys | Ala | Leu 270 | Ser | Lys |
| · - | Pro | Asp | Asp -275 | | Gly | Met | Thr | Gly 280 | Ala | | Met | Asp | Lys - 285 | Phe | Arg | Gln |
| 30 | Ala | Met 290 | | Met | Ile | . Lys | Ser 295 | | val | Ala | Gly | 300 | Thr | Gly | Asn | Thr |
| · | Asr 305 | | ı Asr | Leu | Arg | 310 | | Gl3 | / Gly | / Ala | 315 | Leu 5 | a Gly | , Ile | Asp | Ala 320 |
| | Ala | a Val | Val | Gly | 7 Asp 325 | | s Il€ | e Ala | a Ası | 1 Met | ser | . Let | ı Gly | / Lys | 335 | Ala |
| 35 | Ası | n Ala | ì | | | | | | | | | | | | | |

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains

substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

| 5 | CGATTTTACC | CGGGTGAACG | TGCTATGACC | GACAGCATCA | CGGTATTCGA | CACCGTTACG | 60 |
|----|------------|--------------|-------------|--------------|--------------|--------------|------|
| | GCGTTTATGG | CCGCGATGAA | CCGGCATCAG | GCGGCGCGCT | GGTCGCCGCA | ATCCGGCGTC | 120 |
| | GATCTGGTAT | TTCAGTTTGG | GGACACCGGG | CGTGAACTCA | TGATGCAGAT | TCAGCCGGGG | 180 |
| | CAGCAATATC | CCGGCATGTT | GCGCACGCTG | CTCGCTCGTC | GTTATCAGCA | GGCGGCAGAG | 240 |
| | TGCGATGGCT | GCCATCTGTG | CCTGAACGGC | AGCGATGTAT | TGATCCTCTG | GTGGCCGCTG | 300 |
| 10 | CCGTCGGATC | CCGGCAGTTA | TCCGCAGGTG | ATCGAACGTT | TGTTTGAACT | GGCGGGAATG | 360 |
| | ACGTTGCCGT | CGCTATCCAT | AGCACCGACG | GCGCGTCCGC | AGACAGGGAA | CGGACGCGCC | 420 |
| | CGATCATTAA | GATAAAGGCG | GCTTTTTTA | TTGCAAAACG | GTAACGGTGA | GGAACCGTTT | 480 |
| | CACCGTCGGC | GTCACTCAGT | AACAAGTATC | CATCATGATG | CCTACATCGG | GATCGGCGTG | 540 |
| | GGCATCCGTT | GCAGATACTT | TTGCGAACAC | CTGACATGAA | TGAGGAAACG | AAATTATGCA | 600 |
| 15 | AATTACGATC | AAAGCGCACA | TCGGCGGTGA | TTTGGGCGTC | TCCGGTCTGG | GGCTGGGTGC | 660 |
| | TCAGGGACTG | AAAGGACTGA | ATTCCGCGGC | TTCATCGCTG | GGTTCCAGCG | TGGATAAACT | 720 |
| | GAGCAGCACC | ATCGATAAGT | TGACCTCCGC | GCTGACTTCG | ATGATGTTTG | GCGGCGCGCT | 780 |
| | GGCGCAGGGG | CTGGGCGCCA | GCTCGAAGGG | GCTGGGGATG | AGCAATCAAC | TGGGCCAGTC | 840 |
| | TTTCGGCAAT | GGCGCGCAGG | GTGCGAGCAA | CCTGCTATCC | GTACCGAAAT | CCGGCGGCGA | 900 |
| 20 | TGCGTTGTCA | AAAATGTTTG | ATAAAGCGCT | GGACGATCTG | CTGGGTCATG | ACACCGTGAC | 960 |
| | CAAGCTGACT | AACCAGAGCA | ACCAACTGGC | TAATTCAATG | CTGAACGCCA | GCCAGATGAC | 1020 |
| | CCAGGGTAAT | ATGAATGCGT | TCGGCAGCGG | TGTGAACAAC | GCACTGTCGT | CCATTCTCGG | 1080 |
| | CAACGGTCTC | GGCCAGTCGA | TGAGTGGCT | CTCTCAGCCT | TCTCTGGGGG | CAGGCGGCTT | 1140 |
| | GCAGGGCCTG | AGCGGCGCGG | GTGCATTCA | A CCAGTTGGGT | r AATGCCATCO | GCATGGGCGT | 1200 |
| 25 | GGGGCAGAAT | GCTGCGCTGA | GTGCGTTGAG | TAACGTCAG | C ACCCACGTA | ACGGTAACAA | 1260 |
| | CCGCCACTT | GTAGATAAA | AAGATCGCG | G CATGGCGAA | A GAGATCGGC | CAGTTTATGGA | 1320 |
| | TCAGTATCC | GAAATATTC | GTAAACCGG | A ATACCAGAA | A GATGGCTGG | A GTTCGCCGAA | 1380 |
| | GACGGACGAC | C AAATCCTGGG | CTAAAGCGC | T GAGTAAACC | G GATGATGAC | G GTATGACCGG | 1440 |
| | CGCCAGCATC | GACAAATTC | C GTCAGGCGA | T GGGTATGAT | C AAAAGCGCG | G TGGCGGGTGA | 1500 |
| 30 | TACCGGCAA | r accaacctg | A ACCTGCGTG | G CGCGGGCGG | T GCATCGCTG | G GTATCGATGC | 1560 |
| | GGCTGTCGT | C GGCGATAAA | A TAGCCAACA | т стссстссс | T AAGCTGGCC | A ACGCCTGATA | 1620 |

| | ATCTGTGCTG | GCCTGATAAA | GCGGAAACGA | AAAAAGAGAC | GGGGAAGCCT | GTCTCTTTTC | 1680 |
|---|------------|------------|------------|------------|------------|------------|------|
| | TTATTATGCG | GTTTATGCGG | TTACCTGGAC | CGGTTAATCA | TCGTCATCGA | TCTGGTACAA | 1740 |
| | ACGCACATTT | TCCCGTTCAT | TCGCGTCGTT | ACGCGCCACA | ATCGCGATGG | CATCTTCCTC | 1800 |
| | GTCGCTCAGA | TTGCGCGGCT | GATGGGGAAC | GCCGGGTGGA | ATATAGAGAA | ACTCGCCGGC | 1860 |
| ; | CAGATGGAGA | CACGTCTGCG | ATAAATCTGT | GCCGTAACGT | GTTTCTATCC | GCCCCTTTAG | 1920 |
| | CAGATAGATT | GCGGTTTCGT | AATCAACATG | GTAATGCGGT | TCCGCCTGTG | CGCCGGCCGG | 1980 |
| | GATCACCACA | ATATTCATAG | AAAGCTGTCT | TGCACCTACC | GTATCGCGGG | AGATACCGAC | 2040 |
| | AAAATAGGGC | AGTTTTTGCG | TGGTATCCGT | GGGGTGTTCC | GGCCTGACAA | TCTTGAGTTG | 2100 |
| | GTTCGTCATC | ATCTTTCTCC | ATCTGGGCGA | CCTGATCGGT | T | | 2141 |
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The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

| 15 | Met 1 | Ser | Leu | Asn | Thr 5 | Ser | Gly | Leu | Gly | Ala 10 | Ser | Thr | Met | Gln | Ile 15 | Ser |
|----|------------|------------|-----------|------------|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| | Ile | Gly | Gly | Ala 20 | Gly | Gly | Asn | Asn | Gly 25 | Leu | Leu | Gly | Thr | Ser 30 | Arg | Gl'n |
| 20 | Asn | Ala | Gly 35 | Leu | Gly | Gly | Asn | Ser 40 | Ala | Leu | Gly | Leu | Gly 45 | Gly | Gly | Asn |
| | Gln | Asn 50 | Asp | Thr | Val | Asn | Gln 55 | Leu | Ala | Gly | Leu | Leu 60 | Thr | Gly | Met | Met |
| 25 | Met 65 | Met | Met | Ser | Met | Met 70 | Gly | Gly | Gly | Gly | Leu 75 | Met | Gly | Gly | Gly | Leu 80 |
| | Gly | Gly | Gly | Leu | Gly 85 | Asn | Gly | Leu | Gly | Gly 90 | Ser | Gly | Gly | Leu | Gly 95 | Glu |
| | Gly | Leu | Ser | Asn 100 | | Leu | Asn | Asp | Met 105 | Leu | Gly | Gly | Ser | Leu 110 | Asn | Thr |
| 30 | Leu | Gly | Ser | | Gly | Gly | Asn | Asn 120 | Thr | Thr | Ser | Thr | Thr 125 | Asn | Ser | Pro |
| | Leu | Asp 130 | | Ala | Leu | Gly | Ile 135 | Asn | Ser | Thr | Ser | Gln 140 | Asn | Asp | Asp | Ser |
| 35 | Thr 145 | | Gly | Thr | Asp | Ser 150 | | Ser | Asp | Ser | Ser 155 | Asp | Pro | Met | Gln | Gln 160 |
| | Lev | Leu | Lys | . Met | Phe 165 | ser | Glu | ı Ile | e Met | Glr 170 | ser | Leu | Phe | gly | Asp 175 | Gly |

| | Gln | Asp | Gly | Thr 180 | Gln | Gly | Ser | Ser | Ser 185 | Gly | Gly | Lys | Gln | Pro 190 | Thr | Glu |
|----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| | Gly | Glu | Gln 195 | Asn | Ala | Tyr | Lys | Lys 200 | Gly | Val | Thr | Asp | Ala 205 | Leu | Ser | Gly |
| 5 | Leu | Met 210 | Gly | Asn | Gly | Leu | Ser 215 | Gln | Leu | Leu | Gly | Asn 220 | Gly | Gly | Leu | Gly |
| | Gly 225 | Gly | Gln | Gly | Gly | Asn 230 | Ala | Gly | Thr | Gly | Leu 235 | Asp | Gly | Ser | Ser | Leu 240 |
| 10 | Gly | Gly | Lys | Gly | Leu 245 | Gln | Asn | Leu | Ser | Gly 250 | Pro | Val | Asp | Tyr | Gln 255 | Gln |
| | Leu | Gly | Asn | Ala 260 | Val | Gly | Thr | Gly | Ile 265 | Gly | Met | Lys | Ala | Gly 270 | Ile | Gln |
| | Ala | Leu | Asn 275 | Asp | Ile | Gly | Thr | His 280 | | His | Ser | Ser | Thr 285 | Arg | Ser | Phe |
| 15 | Val | Asn 290 | Lys | Gly | Asp | Arg | Ala 295 | Met | Ala | Lys | Glu | Ile 300 | Gly | Gln | Phe | Met |
| | Asp 305 | Gln | Tyr | Pro | Glu | Val 310 | Phe | Gly | Lys | Pro | Gln 315 | туr | Gln | Lys | Gly | Pro 320 |
| 20 | Gly | Gln | Glu | Val | Lys 325 | Thr | Asp | Asp | Lys | Ser 330 | Trp | Ala | Lys | Ala | Leu 335 | Ser |
| | Lys | Pro | Asp | Asp 340 | Asp | Gly | Met | Thr | Pro 345 | Ala | Ser | Met | Glu | Gln 350 | Phe | Asn |
| | Lys | Ala | Lys 355 | Gly | Met | Ile | Lys | Arg 360 | Pro | Met | Ala | Gly | Asp 365 | Thr | Gly | Asn |
| 25 | Gly | Asn 370 | Leu | Gln | Ala | Arg | Gly 375 | Ala | Gly | Gly | Ser | Ser 380 | Leu | Gly | Ile | Asp |
| | Ala 385 | Met | Met | Ala | Gly | Asp 390 | Ala | Ile | Asn | Asn | Met 395 | Ala | Leu | Gly | Lys | Leu 400 |
| | Gly | Ala | Ala | | | | | | | | | | | | | |

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from Erwinia amylovora is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen Erwinia amylovora,"

Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

| 5 | AAGCTTCGGC | ATGGCACGTT | TGACCGTTGG | GTCGGCAGGG | TACGTTTGAA | TTATTCATAA | 60 |
|----|------------|------------|------------|------------|------------|------------|------|
| | GAGGAATACG | TTATGAGTCT | GAATACAAGT | GGGCTGGGAG | CGTCAACGAT | GCAAATTTCT | 120 |
| | ATCGGCGGTG | CGGGCGGAAA | TAACGGGTTG | CTGGGTACCA | GTCGCCAGAA | TGCTGGGTTG | 180 |
| | GGTGGCAATT | CTGCACTGGG | GCTGGGCGGC | GGTAATCAAA | ATGATACCGT | CAATCAGCTG | 240 |
| | GCTGGCTTAC | TCACCGGCAT | GATGATGATG | ATGAGCATGA | TGGGCGGTGG | TGGGCTGATG | 300 |
| 10 | GGCGGTGGCT | TAGGCGGTGG | CTTAGGTAAT | GGCTTGGGTG | GCTCAGGTGG | CCTGGGCGAA | 360 |
| | GGACTGTCGA | ACGCGCTGAA | CGATATGTTA | GGCGGTTCGC | TGAACACGCT | GGGCTCGAAA | 420 |
| | GGCGGCAACA | ATACCACTTC | AACAACAAAT | TCCCCGCTGG | ACCAGGCGCT | GGGTATTAAC | 480 |
| | TCAACGTCCC | AAAACGACGA | TTCCACCTCC | GGCACAGATT | CCACCTCAGA | CTCCAGCGAC | 540 |
| | CCGATGCAGC | AGCTGCTGAA | GATGTTCAGC | GAGATAATGC | AAAGCCTGTT | TGGTGATGGG | 600 |
| 15 | CAAGATGGCA | CCCAGGGCAG | TTCCTCTGGG | GGCAAGCAGC | CGACCGAAGG | CGAGCAGAAC | 660 |
| | GCCTATAAAA | AAGGAGTCAC | TGATGCGCTG | TCGGGCCTGA | TGGGTAATGG | TCTGAGCCAG | 720 |
| | CTCCTTGGCA | ACGGGGGACT | GGGAGGTGGT | CAGGGCGGTA | ATGCTGGCAC | GGGTCTTGAC | 780 |
| | GGTTCGTCGC | TGGGCGGCAA | AGGGCTGCAA | AACCTGAGCG | GGCCGGTGGA | CTACCAGCAG | 840 |
| | TTAGGTAACG | CCGTGGGTAC | CGGTATCGGT | ATGAAAGCGG | GCATTCAGGC | GCTGAATGAT | 900 |
| 20 | ATCGGTACGC | ACAGGCACAG | TTCAACCCGT | TCTTTCGTCA | ATAAAGGCGA | TCGGGCGATG | 960 |
| | GCGAAGGAAA | TCGGTCAGTT | CATGGACCAG | TATCCTGAGG | TGTTTGGCAA | GCCGCAGTAC | 1020 |
| | CAGAAAGGCC | CGGGTCAGGA | GGTGAAAACC | GATGACAAAT | CATGGGCAAA | AGCACTGAGC | 1080 |
| | AAGCCAGATG | ACGACGGAAT | GACACCAGCC | AGTATGGAGC | AGTTCAACAA | AGCCAAGGGC | 1140 |
| | ATGATCAAAA | GGCCCATGGC | GGGTGATACC | GGCAACGGCA | ACCTGCAGGC | ACGCGGTGCC | 1200 |
| 25 | GGTGGTTCTT | CGCTGGGTAT | TGATGCCATG | ATGGCCGGTG | ATGCCATTAA | CAATATGGCA | 1260 |
| | CTTGGCAAGC | TGGGCGCGGC | TTAAGCTT | | | | 1288 |

Another potentially suitable hypersensitive response elicitor from

Erwinia amylovora is disclosed in U.S. Patent Application Serial No. 09/120,927,
which is hereby incorporated by reference. The protein is encoded by a DNA
molecule having a nucleic acid sequence of SEQ. ID. No. 5 as follows:

| | ATGTCAATTC | TTACGCTTAA | CAACAATACC | TCGTCCTCGC | CGGGTCTGTT | CCAGTCCGGG | 60 |
|-----|------------|------------|------------|------------|------------|------------|------|
| 5 | GGGGACAACG | GGCTTGGTGG | TCATAATGCA | AATTCTGCGT | TGGGGCAACA | ACCCATCGAT | 120 |
| 3 | CGGCAAACCA | TTGAGCAAAT | GGCTCAATTA | TTGGCGGAAC | TGTTAAAGTC | ACTGCTATCG | 180 |
| | CCACAATCAG | GTAATGCGGC | AACCGGAGCC | GGTGGCAATG | ACCAGACTAC | AGGAGTTGGT | 240 |
| 10 | AACGCTGGCG | GCCTGAACGG | ACGAAAAGGC | ACAGCAGGAA | CCACTCCGCA | GTCTGACAGT | 300 |
| | CAGAACATGC | TGAGTGAGAT | GGGCAACAAC | GGGCTGGATC | AGGCCATCAC | GCCCGATGGC | 360 |
| 1.5 | CAGGGCGGCG | GGCAGATCGG | CGATAATCCT | TTACTGAAAG | CCATGCTGAA | GCTTATTGCA | 420 |
| 15 | CGCATGATGG | ACGGCCAAAG | CGATCAGTTT | GGCCAACCTG | GTACGGGCAA | CAACAGTGCC | 480 |
| | TCTTCCGGTA | CTTCTTCATC | TGGCGGTTCC | CCTTTTAACG | ATCTATCAGG | GGGGAAGGCC | 540 |
| 20 | CCTTCCGGCA | ACTCCCCTTC | CGGCAACTAC | TCTCCCGTCA | GTACCTTCTC | ACCCCCATCC | 600 |
| | ACGCCAACGT | CCCCTACCTC | ACCGCTTGAT | TTCCCTTCTT | CTCCCACCAA | AGCAGCCGGG | 660 |
| 25 | GGCAGCACGC | CGGTAACCGA | TCATCCTGAC | CCTGTTGGTA | GCGCGGCAT | CGGGGCCGGA | 720 |
| 23 | AATTCGGTGG | CCTTCACCAG | CGCCGGCGCT | AATCAGACGG | TGCTGCATGA | CACCATTACC | 780 |
| | GTGAAAGCGG | GTCAGGTGTT | TGATGGCAAA | GGACAAACCT | TCACCGCCGG | TTCAGAATTA | 840 |
| 30 | GGCGATGGCG | GCCAGTCTGA | AAACCAGAAA | CCGCTGTTTA | TACTGGAAGA | CGGTGCCAGC | 900 |
| | CTGAAAAACG | TCACCATGGG | CGACGACGG | GCGGATGGTA | TTCATCTTTA | CGGTGATGCC | 960 |
| 35 | AAAATAGACA | ATCTGCACGT | CACCAACGTG | GGTGAGGACG | CGATTACCGT | TAAGCCAAAC | 1020 |
| 33 | AGCGCGGGCA | AAAAATCCCA | CGTTGAAATC | ACTAACAGTT | CCTTCGAGCA | CGCCTCTGAC | 1080 |
| | AAGATCCTGC | AGCTGAATGC | CGATACTAAC | CTGAGCGTTG | ACAACGTGAA | GGCCAAAGAC | 1140 |
| 40 | TTTGGTACTT | TTGTACGCAC | TAACGGCGGT | CAACAGGGTA | ACTGGGATCT | GAATCTGAGC | 1200 |
| | CATATCAGCG | CAGAAGACGG | TAAGTTCTCG | TTCGTTAAAA | GCGATAGCGA | GGGGCTAAAC | 1260 |
| 45 | GTCAATACCA | GTGATATCTC | ACTGGGTGAT | GTTGAAAACC | ACTACAAAGT | GCCGATGTCC | 1320 |
| 10 | GCCAACCTGA | AGGTGGCTGA | ATGA | | | | 1344 |

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 6 as follows:

| | Gln | Leu 50 | Leu | Ala | Glu | Leu | Leu 55 | Lys | Ser | Leu | Leu | Ser 60 | Pro | Gln | Ser | Gly |
|-----------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|--------------|-------------|------------|
| 5 | Asn 65 | Ala | Ala | Thr | Gly | Ala 70 | Gly | Gly | Asn | Asp | Gln 75 | Thr | Thr | Gly | Val | Gly 80 |
| | Asn | Ala | Gly | Gly | Leu 85 | Asn | Gly | Arg | Lys | Gly 90 | Thr | Ala | Gly | Thr | Thr 95 | Pro |
| 10 | Gln | Ser | Asp | Ser 100 | Gln | Asn | Met | Leu | Ser 105 | Glu | Met | Gly | Asn | Asn 110 | Gly | Leu |
| 15 | Asp | Gln | Ala 115 | Ile | Thr | Pro | Asp | Gly 120 | Gln | Gly | Gly | Gly | Gln 125 | Ile | Gly | Asp |
| | Asn | Pro 130 | Leu | Leu | Lys | Ala | Met 135 | Leu | Lys | Leu | Ile | Ala 140 | Arg | Met | Met | Asp |
| 20 | Gly 145 | Gln | Ser | Asp | Gln | Phe 150 | Gly | Gln | Pro | Gly | Thr 155 | Gly | Asn | Asn | Ser | Ala 160 |
| 25 | Ser | Ser | Gly | Thr | Ser 165 | Ser | Ser | Gly | Gly | Ser 170 | Pro | Phe | Àsn | Asp | Leu 175 | Ser |
| 25 | Gly | Gly | Lys | Ala 180 | Pro | Ser | Gly | Asn | Ser 185 | Pro | Ser | Gly | Asn | Tyr 190 | Ser | Pro |
| 30 | | | 195 | | | | | 200 | | | | | 205 | | Ser | |
| | | 210 | | | | | 215 | | | | | 220 | ı | | | Pro |
| 35 | 225 | | | | | 230 | | | | | 235 | i | | | | Gly 240 |
| 40 | | | | | 245 | | | | | 250 | | | | | ∠ 55 | |
| 40 | | | | 260 |) | | | | 265 | 5 | | | | 270 | ' | Gln |
| 45 | | | 275 | 5 | | | | 280 |) | | | | 285 | • | | Asn |
| | | 290 |) | | | | 295 | i | | | | 300 | J | | | Val |
| 50 | 305 | 5 | | | | 310 |) | | | | 31! | • | | | | 320 |
| 55 | | | | | 325 | 5 | | | | 330 |) | | | | 335 | |
| <i>JJ</i> | Va] | L Lys | s Pro | 34(| | Ala | a Gly | / Lys | 345 | s Sei | r Hi | s Va | l Glı | ı Il∈ 350 | e Thi | Asn |

| | Ser | Ser | Phe 355 | Glu | His | Ala | Ser | Asp 360 | Lys | Ile | Leu | Gln | Leu 365 | Asn | Ala | Asp |
|-----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 5 | Thr | Asn 370 | Leu | Ser | Val | Asp | Asn 375 | Val | Lys | Ala | Lys | Asp 380 | Phe | Gly | Thr | Phe |
| | Val 385 | Arg | Thr | Asn | Gly | Gly 390 | Gln | Gln | Gly | Asn | Trp 395 | Asp | Leu | Asn | Leu | Ser 400 |
| 10 | His | Ile | Ser | Ala | Glu 405 | Asp | Gly | Lys | Phe | Ser 410 | Phe | Val | Lys | Ser | Asp 415 | Ser |
| 1.5 | Glu | Gly | Leu | Asn 420 | Val | Asn | Thr | Ser | Asp 425 | Ile | Ser | Leu | Gly | Asp 430 | Val | Glu |
| 15 | Asn | His | Tyr 435 | Lys | Val | Pro | Met | Ser 440 | Ala | Asn | Leu | Lys | Val 445 | Ala | Glu | |

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from Erwinia amylovora is disclosed in U.S. Patent Application Serial No. 09/120,663, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 7 as follows:

| 20 | ATGGAATTAA | AATCACTGGG | AACTGAACAC | AAGGCGGCAG | TACACACAGC | GGCGCACAAC | 60 |
|----|------------|------------|------------|------------|------------|------------|-----|
| 30 | CCTGTGGGGC | ATGGTGTTGC | CTTACAGCAG | GGCAGCAGCA | GCAGCAGCCC | GCAAAATGCC | 120 |
| | GCTGCATCAT | TGGCGGCAGA | AGGCAAAAAT | CGTGGGAAAA | TGCCGAGAAT | TCACCAGCCA | 180 |
| 35 | TCTACTGCGG | CTGATGGTAT | CAGCGCTGCT | CACCAGCAAA | AGAAATCCTT | CAGTCTCAGG | 240 |
| | GGCTGTTTGG | GGACGAAAAA | ATTTTCCAGA | TCGGCACCGC | AGGGCCAGCC | AGGTACCACC | 300 |
| 40 | CACAGCAAAG | GGGCAACATT | GCGCGATCTG | CTGGCGCGGG | ACGACGGCGA | AACGCAGCAT | 360 |
| 40 | GAGGCGGCCG | CGCCAGATGC | GGCGCGTTTG | ACCCGTTCGG | GCGGCGTCAA | ACGCCGCAAT | 420 |
| | ATGGACGACA | TGGCCGGGCG | GCCAATGGTG | AAAGGTGGCA | GCGGCGAAGA | TAAGGTACCA | 480 |
| 45 | ACGCAGCAAA | AACGGCATCA | GCTGAACAAT | TTTGGCCAGA | TGCGCCAAAC | GATGTTGAGC | 540 |
| | AAAATGGCTC | ACCCGGCTTC | AGCCAACGCC | GGCGATCGCC | TGCAGCATTC | ACCGCCGCAC | 600 |
| 50 | ATCCCGGGTA | GCCACCACGA | AATCAAGGAA | GAACCGGTTG | GCTCCACCAG | CAAGGCAACA | 660 |
| 30 | ACGGCCCACG | CAGACAGAGT | GGAAATCGCT | CAGGAAGATG | ACGACAGCGA | ATTCCAGCAA | 720 |
| | CTGCATCAAC | AGCGGCTGGC | GCGCGAACGG | GAAAATCCAC | CGCAGCCGCC | CAAACTCGGC | 780 |
| 55 | GTTGCCACAC | CGATTAGCGC | CAGGTTTCAG | CCCAAACTGA | CTGCGGTTGC | GGAAAGCGTC | 840 |

| | CTTGAGGGGA | CAGATACCAC | GCAGTCACCC | CTTAAGCCGC | AATCAATGCT | GAAAGGAAGT | 900 |
|-----|------------|------------|------------|------------|------------|------------|------|
| _ | GGAGCCGGGG | TAACGCCGCT | GGCGGTAACG | CTGGATAAAG | GCAAGTTGCA | GCTGGCACCG | 960 |
| 5 | GATAATCCAC | CCGCGCTCAA | TACGTTGTTG | AAGCAGACAT | TGGGTAAAGA | CACCCAGCAC | 1020 |
| | TATCTGGCGC | ACCATGCCAG | CAGCGACGGT | AGCCAGCATC | TGCTGCTGGA | CAACAAAGGC | 1080 |
| 10 | CACCTGTTTG | ATATCAAAAG | CACCGCCACC | AGCTATAGCG | TGCTGCACAA | CAGCCACCCC | 1140 |
| | GGTGAGATAA | AGGGCAAGCT | GGCGCAGGCG | GGTACTGGCT | CCGTCAGCGT | AGACGGTAAA | 1200 |
| 1.5 | AGCGGCAAGA | TCTCGCTGGG | GAGCGGTACG | CAAAGTCACA | ACAAAACAAT | GCTAAGCCAA | 1260 |
| 15 | CCGGGGGAAG | CGCACCGTTC | CTTATTAACC | GGCATTTGGC | AGCATCCTGC | TGGCGCAGCG | 1320 |
| | CGGCCGCAGG | GCGAGTCAAT | CCGCCTGCAT | GACGACAAAA | TTCATATCCT | GCATCCGGAG | 1380 |
| 20 | CTGGGCGTAT | GGCAATCTGC | GGATAAAGAT | ACCCACAGCC | AGCTGTCTCG | CCAGGCAGAC | 1440 |
| | GGTAAGCTCT | ATGCGCTGAA | AGACAACCGT | ACCCTGCAAA | ACCTCTCCGA | TAATAAATCC | 1500 |
| 25 | TCAGAAAAGC | TGGTCGATAA | AATCAAATCG | TATTCCGTTG | ATCAGCGGGG | GCAGGTGGCG | 1560 |
| 23 | ATCCTGACGG | ATACTCCCGG | CCGCCATAAG | ATGAGTATTA | TGCCCTCGCT | GGATGCTTCC | 1620 |
| | CCGGAGAGCC | ATATTTCCCT | CAGCCTGCAT | TTTGCCGATG | CCCACCAGGG | GTTATTGCAC | 1680 |
| 30 | GGGAAGTCGG | AGCTTGAGGC | ACAATCTGTC | GCGATCAGCC | ATGGGCGACT | GGTTGTGGCC | 1740 |
| | GATAGCGAAG | GCAAGCTGTT | TAGCGCCGCC | ATTCCGAAGC | AAGGGGATGG | AAACGAACTG | 1800 |
| 35 | AAAATGAAAG | CCATGCCTCA | GCATGCGCTC | GATGAACATT | TTGGTCATGA | CCACCAGATT | 1860 |
| 55 | TCTGGATTTT | TCCATGACGA | CCACGGCCAG | CTTAATGCGC | TGGTGAAAAA | TAACTTCAGG | 1920 |
| | CAGCAGCATG | CCTGCCCGTT | GGGTAACGAT | CATCAGTTTC | ACCCCGGCTG | GAACCTGACT | 1980 |
| 40 | GATGCGCTGG | TTATCGACAA | TCAGCTGGGG | CTGCATCATA | CCAATCCTGA | ACCGCATGAG | 2040 |
| | ATTCTTGATA | TGGGGCATTT | AGGCAGCCTG | GCGTTACAGG | AGGGCAAGCT | TCACTATTTT | 2100 |
| 45 | GACCAGCTGA | CCAAAGGGTG | GACTGGCGCG | GAGTCAGATT | GTAAGCAGCT | GAAAAAAGGC | 2160 |
| 43 | CTGGATGGAG | CAGCTTATCT | ACTGAAAGAC | GGTGAAGTGA | AACGCCTGAA | TATTAATCAG | 2220 |
| | AGCACCTCCT | CTATCAAGCA | CGGAACGGAA | AACGTTTTTT | CGCTGCCGCA | TGTGCGCAAT | 2280 |
| 50 | AAACCGGAGC | CGGGAGATGC | CCTGCAAGGG | CTGAATAAAG | ACGATAAGGC | CCAGGCCATG | 2340 |
| | GCGGTGATTG | GGGTAAATAA | ATACCTGGCG | CTGACGGAAA | AAGGGGACAT | TCGCTCCTTC | 2400 |
| 55 | CAGATAAAAC | CCGGCACCCA | GCAGTTGGAG | CGGCCGGCAC | AAACTCTCAG | CCGCGAAGGT | 2460 |
| | ATCAGCGGCG | AACTGAAAGA | CATTCATGTC | GACCACAAGC | AGAACCTGTA | TGCCTTGACC | 2520 |
| | CACGAGGGAG | AGGTGTTTCA | TCAGCCGCGT | GAAGCCTGGC | AGAATGGTGC | CGAAAGCAGC | 2580 |
| 60 | | | | | | GGACATGAGC | |
| | CATGAGCACA | AACCGATTGC | CACCTTTGAA | GACGGTAGCC | AGCATCAGCT | GAAGGCTGGC | 2700 |
| 65 | GGCTGGCACG | CCTATGCGGC | ACCTGAACGC | GGGCCGCTGG | CGGTGGGTAC | CAGCGGTTCA | 2760 |

| | CAAACCGTCT | TTAACCGACT | AATGCAGGGG | GTGAAAGGCA | AGGTGATCCC | AGGCAGCGGG | 2820 |
|----------------|------------|------------|------------|------------|------------|------------|------|
| | TTGACGGTTA | AGCTCTCGGC | TCAGACGGGG | GGAATGACCG | GCGCCGAAGG | GCGCAAGGTC | 2880 |
| 5 | AGCAGTAAAT | TTTCCGAAAG | GATCCGCGCC | TATGCGTTCA | ACCCAACAAT | GTCCACGCCG | 2940 |
| | CGACCGATTA | AAAATGCTGC | TTATGCCACA | CAGCACGGCT | GGCAGGGGCG | TGAGGGGTTG | 3000 |
| 10 | AAGCCGTTGT | ACGAGATGCA | GGGAGCGCTG | ATTAAACAAC | TGGATGCGCA | TAACGTTCGT | 3060 |
| 10 | CATAACGCGC | CACAGCCAGA | TTTGCAGAGC | AAACTGGAAA | CTCTGGATTT | AGGCGAACAT | 3120 |
| | GGCGCAGAAT | TGCTTAACGA | CATGAAGCGC | TTCCGCGACG | AACTGGAGCA | GAGTGCAACC | 3180 |
| 15 | CGTTCGGTGA | CCGTTTTAGG | TCAACATCAG | GGAGTGCTAA | AAAGCAACGG | TGAAATCAAT | 3240 |
| | AGCGAATTTA | AGCCATCGCC | CGGCAAGGCG | TTGGTCCAGA | GCTTTAACGT | CAATCGCTCT | 3300 |
| 20 | GGTCAGGATC | TAAGCAAGTC | ACTGCAACAG | GCAGTACATG | CCACGCCGCC | ATCCGCAGAG | 3360 |
| 20 | AGTAAACTGC | AATCCATGCT | GGGGCACTTT | GTCAGTGCCG | GGGTGGATAT | GAGTCATCAG | 3420 |
| | AAGGGCGAGA | TCCCGCTGGG | CCGCCAGCGC | GATCCGAATG | ATAAAACCGC | ACTGACCAAA | 3480 |
| 25 | TCGCGTTTAA | TTTTAGATAC | CGTGACCATC | GGTGAACTGC | ATGAACTGGC | CGATAAGGCG | 3540 |
| | AAACTGGTAT | CTGACCATAA | ACCCGATGCC | GATCAGATAA | AACAGCTGCG | CCAGCAGTTC | 3600 |
| 20 | GATACGCTGC | GTGAAAAGCG | GTATGAGAGC | AATCCGGTGA | AGCATTACAC | CGATATGGGC | 3660 |
| 30 | TTCACCCATA | ATAAGGCGCT | GGAAGCAAAC | TATGATGCGG | TCAAAGCCTT | TATCAATGCC | 3720 |
| | TTTAAGAAAG | AGCACCACGG | CGTCAATCTG | ACCACGCGTA | CCGTACTGGA | ATCACAGGGC | 3780 |
| 35 | AGTGCGGAGC | TGGCGAAGAA | GCTCAAGAAT | ACGCTGTTGT | CCCTGGACAG | TGGTGAAAGT | 3840 |
| | ATGAGCTTCA | GCCGGTCATA | TGGCGGGGGC | GTCAGCACTG | TCTTTGTGCC | TACCCTTAGC | 3900 |
| 40 | AAGAAGGTGC | CAGTTCCGGT | GATCCCCGGA | GCCGGCATCA | CGCTGGATCG | CGCCTATAAC | 3960 |
| 40 | CTGAGCTTCA | GTCGTACCAG | CGGCGGATTG | AACGTCAGTT | TTGGCCGCGA | CGGCGGGGTG | 4020 |
| | AGTGGTAACA | TCATGGTCGC | TACCGGCCAT | GATGTGATGC | CCTATATGAC | CGGTAAGAAA | 4080 |
| 45 | ACCAGTGCAG | GTAACGCCAG | TGACTGGTTG | AGCGCAAAAC | ATAAAATCAG | CCCGGACTTG | 4140 |
| | CGTATCGGCG | CTGCTGTGAG | TGGCACCCTG | CAAGGAACGC | TACAAAACAG | CCTGAAGTTT | 4200 |
| 50 | AAGCTGACAG | AGGATGAGCT | GCCTGGCTTT | ATCCATGGCT | TGACGCATGG | CACGTTGACC | 4260 |
| 30 | CCGGCAGAAC | TGTTGCAAAA | GGGGATCGAA | CATCAGATGA | AGCAGGGCAG | CAAACTGACG | 4320 |
| | TTTAGCGTCG | ATACCTCGGC | AAATCTGGAT | CTGCGTGCCG | GTATCAATCT | GAACGAAGAC | 4380 |
| 55 | GGCAGTAAAC | CAAATGGTGT | CACTGCCCGT | GTTTCTGCCG | GGCTAAGTGC | ATCGGCAAAC | 4440 |
| | CTGGCCGCCG | GCTCGCGTGA | ACGCAGCACC | ACCTCTGGCC | AGTTTGGCAG | CACGACTTCG | 4500 |
| 60 | GCCAGCAATA | ACCGCCCAAC | CTTCCTCAAC | GGGGTCGGCG | CGGGTGCTAA | CCTGACGGCT | 4560 |
| U U | GCTTTAGGGG | TTGCCCATTC | ATCTACGCAT | GAAGGGAAAC | CGGTCGGGAT | CTTCCCGGCA | 4620 |
| | TTTACCTCGA | CCAATGTTTC | GGCAGCGCTG | GCGCTGGATA | ACCGTACCTC | ACAGAGTATC | 4680 |
| 65 | AGCCTGGAAT | TGAAGCGCGC | GGAGCCGGTG | ACCAGCAACG | ATATCAGCGA | GTTGACCTCC | 4740 |

| | ACGCTGGGAA | AACACTTTAA | GGATAGCGCC | ACAACGAAGA | TGCTTGCCGC | TCTCAAAGAG | 4800 |
|----|------------|------------|------------|------------|------------|------------|------|
| 5 | TTAGATGACG | CTAAGCCCGC | TGAACAACTG | CATATTTTAC | AGCAGCATTT | CAGTGCAAAA | 4860 |
| 3 | GATGTCGTCG | GTGATGAACG | CTACGAGGCG | GTGCGCAACC | TGAAAAAACT | GGTGATACGT | 4920 |
| | CAACAGGCTG | CGGACAGCCA | CAGCATGGAA | TTAGGATCTG | CCAGTCACAG | CACGACCTAC | 4980 |
| 10 | AATAATCTGT | CGAGAATAAA | TAATGACGGC | ATTGTCGAGC | TGCTACACAA | ACATTTCGAT | 5040 |
| | GCGGCATTAC | CAGCAAGCAG | TGCCAAACGT | CTTGGTGAAA | TGATGAATAA | CGATCCGGCA | 5100 |
| 15 | CTGAAAGATA | TTATTAAGCA | GCTGCAAAGT | ACGCCGTTCA | GCAGCGCCAG | CGTGTCGATG | 5160 |
| 13 | GAGCTGAAAG | ATGGTCTGCG | TGAGCAGACG | GAAAAAGCAA | TACTGGACGG | TAAGGTCGGT | 5220 |
| | CGTGAAGAAG | TGGGAGTACT | TTTCCAGGAT | CGTAACAACT | TGCGTGTTAA | ATCGGTCAGC | 5280 |
| 20 | GTCAGTCAGT | CCGTCAGCAA | AAGCGAAGGC | TTCAATACCC | CAGCGCTGTT | ACTGGGGACG | 5340 |
| | AGCAACAGCG | CTGCTATGAG | CATGGAGCGC | AACATCGGAA | CCATTAATTT | TAAATACGGC | 5400 |
| 25 | CAGGATCAGA | ACACCCCACG | GCGATTTACC | CTGGAGGGTG | GAATAGCTCA | GGCTAATCCG | 5460 |
| 23 | CAGGTCGCAT | CTGCGCTTAC | TGATTTGAAG | AAGGAAGGGC | TGGAAATGAA | GAGCTAA | 5517 |

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 8 as follows:

| 35 | Met 1 | Glu | Leu | Lys | Ser 5 | Leu | Gly | Thr | Glu | His 10 | Lys | Ala | Ala | Val | His 15 | Thr |
|----|-----------|------------|------------|------------|-----------|-----------|------------|------------|------------|-----------|-----------|------------|------------|------------|-----------|-----------|
| | Ala | Ala | His | Asn 20 | Pro | Val | Gly | His | Gly 25 | Val | Ala | Leu | Gln | Gln 30 | Gly | Ser |
| 40 | Ser | Ser | Ser | Ser | Pro | Gln | Asn | Ala 40 | Ala | Ala | Ser | Leu | Ala 45 | Ala | Glu | Gly |
| 45 | Lys | Asn 50 | Arg | Gly | Lys | Met | Pro 55 | Arg | Ile | His | Gln | Pro 60 | Ser | Thr | Ala | Ala |
| 13 | Asp 65 | Gly | Ile | Ser | Ala | Ala 70 | His | Gln | Gln | Lys | Lys 75 | Ser | Phe | Ser | Leu | Arg 80 |
| 50 | Gly | Cys | Leu | Gly | Thr 85 | Lys | Lys | Phe | Ser | Arg 90 | Ser | Ala | Pro | Gln | Gly 95 | Gln |
| | Pro | Gly | Thr | Thr 100 | His | Ser | Lys | Gly | Ala 105 | Thr | Leu | Arg | Asp | Leu 110 | Leu | Ala |
| 55 | Arg | Asp | Asp 115 | Gly | Glu | Thr | Gln | His 120 | Glu | Ala | Ala | Ala | Pro 125 | Asp | Ala | Ala |
| 60 | Arg | Leu 130 | Thr | Arg | Ser | Gly | Gly 135 | Val | Lys | Arg | Arg | Asn 140 | Met | Asp | Asp | Met |

WO 00/28055 PCT/US99/26039

| | | Ala 145 | Gly | Arg | Pro | Met | Val 150 | Lys | Gly | Gly | Ser | Gly 155 | Glu | Asp | Lys | Val | Pro |
|----|---|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 5 | | Thr | Gln | Gln | Lys | Arg 165 | His | Gln | Leu | Asn | Asn 170 | Phe | Gly | Gln | Met | Arg 175 | Gln |
| | • | Thr | Met | Leu | Ser 180 | Lys | Met | Ala | His | Pro 185 | Ala | Ser | Ala | Asn | Ala 190 | Gly | Asp |
| 10 | | Arg | Leu | Gln 195 | His | Ser | Pro | Pro | His 200 | Ile | Pro | Gly | Ser | His 205 | His | Glu | Ile |
| 15 | | Lys | Glu 210 | Glu | Pro | Val | Gly | Ser 215 | Thr | Ser | Lys | Ala | Thr 220 | Thr | Ala | His | Ala |
| 13 | | Asp 225 | Arg | Val | Glu | Ile | Ala 230 | Gln | Glu | Asp | Asp | Asp 235 | Ser | Glu | Phe | Gln | Gln 240 |
| 20 | | Leu | His | Gln | Gln | Arg 245 | Leu | Ala | Arg | Glu | Arg 250 | Glu | Asn | Pro | Pro | Gln 255 | Pro |
| | | Pro | Lys | Leu | Gly 260 | Val | Ala | Thr | Pro | 11e 265 | Ser | Ala | Arg | Phe | Gln 270 | Pro | Lys |
| 25 | | Leu | Thr | Ala 275 | Val | Ala | Glu | Ser | Val 280 | Leu | Glu | Gly | Thr | Asp 285 | Thr | Thr | Gln |
| 30 | | Ser | Pro 290 | Leu | Lys | Pro | Gln | Ser 295 | Met | Leu | Lys | Gly | Ser 300 | Gly | Ala | Gly | Val |
| 50 | | Thr 305 | Pro | Leu | Ala | Val | Thr 310 | Leu | Asp | Lys | Gly | Lys 315 | Leu | Gln | Leu | Ala | Pro 320 |
| 35 | | Asp | Asn | Pro | Pro | Ala 325 | Leu | Asn | Thr | Leu | Leu 330 | Lys | Gln | Thr | Leu | Gly 335 | Lys |
| | | Asp | Thr | Gln | His 340 | Tyr | Leu | Ala | His | His 345 | Ala | Ser | Ser | Asp | Gly 350 | Ser | Gln |
| 40 | | His | Leu | Leu 355 | Leu | Asp | Asn | Lys | Gly 360 | His | Leu | Phe | Asp | Ile 365 | Lys | Ser | Thr |
| 45 | | Ala | Thr 370 | Ser | Tyr | Ser | Val | Leu 375 | His | Asn | Ser | His | Pro 380 | Gly | Glu | Ile | Lys |
| | | Gly 385 | Lys | Leu | Ala | Gln | Ala 390 | Gly | Thr | Gly | Ser | Val 395 | Ser | Val | Asp | Gly | Lys 400 |
| 50 | | Ser | Gly | Lys | Ile | Ser 405 | Leu | Gly | Ser | Gly | Thr 410 | Gln | Ser | His | Asn | Lys 415 | Thr |
| | | Met | Leu | Ser | Gln 420 | Pro | Gly | Glu | Ala | His 425 | Arg | Ser | Leu | Leu | Thr 430 | Gly | Ile |
| 55 | | Trp | Gln | His 435 | Pro | Ala | Gly | Ala | Ala 440 | Arg | Pro | Gln | Gly | Glu 445 | Ser | Ile | Arg |
| 60 | ı | Leu | His 450 | Asp | Asp | Lys | Ile | His 455 | Ile | Leu | His | Pro | Glu 460 | Leu | Gly | Val | Trp |
| | | Gln 465 | Ser | Ala | Asp | Lys | Asp 470 | Thr | His | Ser | Gln | Leu 475 | Ser | Arg | Gln | Ala | Asp 480 |
| 65 | | Gly | Lys | Leu | Tyr | Ala 485 | Leu | Lys | Asp | Asn | Arg 490 | Thr | Leu | Gln | Asn | Leu 495 | Ser |

| | Asp | Asn | Lys | Ser 500 | Ser | Glu | Lys | Leu | Val 505 | Asp | Lys | Ile | Lys | Ser 510 | Tyr | Ser |
|------------|------------|------------|------------|--------------|------------|--------------|------------|--------------|-------------|------------|------------|------------|--------------|------------|------------|------------|
| 5 | Val | Asp | Gln 515 | Arg | Gly | Gln | Val | Ala 520 | Ile | Leu | Thr | Asp | Thr 525 | Pro | Gly | Arg |
| | His | Lys 530 | Met | Ser | Ile | Met | Pro 535 | Ser | Leu | Asp | Ala | Ser 540 | Pro | Glu | Ser | His |
| 10 | Ile 545 | Ser | Leu | Ser | Leu | His 550 | Phe | Ala | Asp | Ala | His 555 | Gln | Gly | Leu | Leu | His 560 |
| 15 . | Gly | Lys | Ser | Glu | Leu 565 | Glu | Ala | Gln | Ser | Val 570 | Ala | Ile | Ser | His | Gly 575 | Arg |
| 15 | Leu | Val | Val | Ala 580 | Asp | Ser | Glu | Gly | Lys 585 | Leu | Phe | Ser | Ala | Ala 590 | Ile | Pro |
| 20 | Lys | Gln | Gly 595 | Asp | Gly | Asn | Glu | Leu 600 | Lys | Met | Lys | Ala | Met 605 | Pro | Gln | His |
| | Ala | Leu 610 | Asp | Glu | His | Phe | Gly 615 | His | Asp | His | Gln | Ile 620 | Ser | Gly | Phe | Phe |
| 25 | 625 | | | | • | 630 | | | | | 635 | | | | | 640 |
| 30 | | Gln | | | 645 | | | | | 650 | | | | | 655 | |
| 30 | | | | 660 | | | | | 665 | | | | | 670 | | His |
| 35 | | | 675 | | | | | 680 | | | | | 685 | | | Gly |
| | | 690 | | | | | 695 | | | | | 700 | | | | Thr |
| 40 | 705 | ; | | | | 710 |) | | | | 715 | | | | | Gly 720 |
| 45 | | | | | 725 | 5 | | | | 730 |) | | | | 735 | |
| 4 5 | Asn | ılle | Asn | 740 | | Thr | Ser | Ser | 745 | | His | Gly | Thr | 750 | Asn | Val |
| 50 | | | 755 | ; | | | | 760 | | | | | 765 | • | | Leu |
| | | 770 |) | | | | 775 | i | | | | 780 |) | | | Gly |
| 55 | Va] 789 | | Lys | туг | Leu | 1 Ala 790 | | Thr | Glu | ı Lys | 795 | Asp |) Ile | Arg | , Ser | 800 |
| 60 | Glr | ı Ile | Lys | s Pro | 805 | | c Glr | ı Glr | ı Lev | 3 Gl | | g Pro | Ala | a Glr | 815 | Leu |
| oo | Sei | r Arg | g Glu | 1 Gly 820 | | e Sei | c Gly | / Glu | 1 Let 82 | u Ly: 5 | s Asp | o Ile | e His | 830 | L Asp | His |
| 65 | Lys | s Glr | Ası 839 | | туз | r Ala | a Lev | 1 Thi 840 | | s Gl | u Gly | / Glu | ı Va. 84! | l Phe | e His | Gln |

| | Pro | Arg 850 | Glu | Ala | Trp | Gln | Asn 855 | Gly | Ala | Glu | Ser | Ser 860 | Ser | Trp | His | Lys |
|----------------------|--------------------------------------|---|---|---|---|--------------------------------------|---------------------------------------|---|--|--|--------------------------------------|---------------------------------|---|---|---|----------------------------------|
| 5 | Leu 865 | Ala | Leu | Pro | Gln | Ser 870 | Glu | Ser | Lys | Leu | Lys 8 7 5 | Ser | Leu | Asp | Met | Ser 880 |
| • | His | Glu | His | Lys | Pro 885 | Ile | Ala | Thr | Phe | Glu 890 | Asp | Gly | Ser | Gln | His 895 | Gln |
| 10 | Leu | Lys | Ala | Gly 900 | Gly | Trp | His | Ala | Tyr 905 | Ala | Ala | Pro | Glu | Arg 910 | Gly | Pro |
| 15 | Leu | Ala | Val 915 | Gly | Thr | Ser | Gly | Ser 920 | Gln | Thr | Val | Phe | Asn 925 | Arg | Leu | Met |
| 15 | Gln | Gly 930 | Val | Lys | Gly | Lys | Val 935 | Ile | Pro | Gly | Ser | Gly 940 | Leu | Thr | Val | Lys |
| 20 | Leu 945 | Ser | Ala | Gln | Thr | Gly 950 | Gly | Met | Thr | Gly | Ala 955 | Glu | Gly | Arg | Lys | Val 960 |
| | Ser | Ser | Lys | Phe | Ser 965 | Glu | Arg | Ile | Arg | Ala 970 | Tyr | Ala | Phe | Asn | Pro 975 | Thr |
| 25 | Met | Ser | Thr | Pro 980 | Arg | Pro | Ile | Lys | Asn 985 | Ala | Ala | Tyr | Ala | Thr 990 | Gln | His |
| 20 | Gly | Trp | Gln 995 | Gly | Arg | Glu | Gly | Leu 1000 | | Pro | Leu | Tyr | Glu 1009 | | Gln | Gly |
| 30 | Ala | Leu 1010 | | Lys | Gln | Leu | Asp 1019 | | His | Asn | Val | Arg 1020 | | Asn | Ala | Pro |
| | | | | | | | | | | | | | | | | |
| 35 | Gln 1029 | | Asp | Leu | Gln | Ser 103 | | Leu | Glu | Thr | Leu 103 | | Leu | Gly | Glu | His 1040 |
| 35 | 1029 | | | | | 1036 Asn | כ | | | | 1039 Phe | 5 | | | | 1040 Glu |
| 35 | 1029 Gly | 5 | Glu | Leu | Leu 1049 Arg | 1036 Asn | Asp | Met | Lys | Arg 1050 Leu | 1039 Phe | Arg | Asp | Glu | Leu 1059 | 1040 Glu |
| 40 | Gly Gln | Ala | Glu Ala | Leu Thr 1060 | Leu 1049 Arg | 1036 Asn 5 Ser | Asp Val | Met Thr | Lys Val 1069 | Arg 1050 Leu 5 | Phe C Gly | Arg Gln | Asp His | Glu Gln 107 | Leu 105! Gly 0 | 1040 Glu F Val |
| | Gly Gln Leu | Ala Ser | Glu Ala Ser 1079 | Thr 1060 Asn | Leu 1049 Arg O | Asn Ser | Asp Val Ile | Met Thr Asn 1086 | Lys Val 1069 Ser | Arg 1050 Leu 5 | Phe O Gly Phe Arg | Arg Gln Lys | Asp His Pro 1089 | Glu Gln 107 Ser | Leu 1059 Gly Pro | Glu Glu Val |
| 40 | Gly Gln Leu Lys | Ala Ser Lys Ala 1090 | Glu Ala Ser 1079 | Thr 1066 Asn 5 | Leu 1049 Arg O Gly | Asn Ser Glu Ser | Asp Val Ile Phe 1099 | Met Thr Asn 1080 Asn | Val 1069 Ser Val | Arg 1050 Leu 5 Glu Asn | Phe Gly Phe Arg | Arg Gln Lys Ser 110 | Asp His Pro 1089 Gly | Glu Gln 107 Ser 5 | Leu 1059 Gly Pro | Glu Glu Val |
| 40 45 | Gly Gln Leu Lys Ser | Ala Ser Lys Ala 1090 | Glu Ala Ser 1079 Leu Ser | Thr 1060 Asn Val | Leu 1049 Arg Gly Gln Gln | Asn Ser Glu Ser Gln 1110 | Asp Val Ile Phe 1099 | Met Thr Asn 1080 Asn 5 | Val 1069 Ser Val | Arg 1050 Leu 5 Glu Asn Ala | Phe Gly Phe Arg Thr 111: | Arg Gln Lys Ser 110 | Asp His Pro 1089 Gly O | Glu Gln 107 Ser Gln Ser | Leu 1059 Gly Pro Asp | Glu Val Gly Leu Glu 1120 Asp |
| 40 45 | Gly Gln Leu Lys Ser 1105 | Ala Ser Lys Ala 1090 Lys | Glu Ala Ser 1079 Leu Ser | Thr 1060 Asn Val Leu | Leu 1049 Arg Gly Gln Gln Ser 1129 | Asn Ser Glu Ser Gln 1116 | Asp Val Ile Phe 1099 Ala 0 | Met Thr Asn 1080 Asn 5 | Val 1069 Ser Val His | Arg 1050 Leu 5 Glu Asn Ala Phe 113 | Phe Gly Phe Arg Thr | Arg Gln Lys Ser 110 Pro Ser | Asp His Pro 1089 Gly Pro Ala | Glu Gln 107 Ser Gln Gln Gln | Leu 1059 Gly Pro Asp Ala Val 113 | Glu Val Gly Leu Glu 1120 Asp |
| 40 45 50 55 | Gly Gln Leu Lys Ser 1109 Ser | Ala Ser Lys Ala 1090 Lys Lys | Glu Ala Ser 1079 Leu Ser Leu His | Leu Thr 1060 Asn Val Leu Gln Gln 1140 Thr | Leu 1049 Arg Gly Gln Gln Ser 1129 | Asn Ser Glu Ser Gln inter Gly Gly | Asp Val Ile Phe 1099 Ala O Leu Glu | Met Thr Asn 1080 Asn Val Gly Ile | Val 1069 Ser Val His Pro 1149 | Arg 1050 Leu 5 Glu Asn Ala Phe 113 | Phe Gly Phe Arg Thr 111: Val | Arg Gln Lys Ser 110 Pro Ser Arg | Asp His Pro 108: Gly Pro Ala Gln | Glu Gln 107 Ser Gln Ser Gly Arg 115 | Leu 1059 Gly Pro Asp Ala Val 113 Asp | Glu Val Gly Leu Glu 1120 Asp Pro |
| 40 45 50 | Gly Gln Leu Lys Ser 1105 Ser Met Asn | Ala Ser Lys Ala 1090 Lys Ser Asp | Glu Ala Ser 1079 Leu Ser Leu His Lys 1159 | Leu Thr 1060 Asn Val Leu Gln Gln 1140 Thr | Leu 1049 Arg Gly Gln Gln Ser 1129 Lys | Asn Ser Glu Ser Gln 1110 Met Gly Leu | Asp Val Ile Phe 1099 Ala Cleu Glu Thr | Met Thr Asn 1080 Asn Val Gly Ile Lys 1160 Leu | Val 1069 Ser Val His Pro 1149 Ser | Arg 1050 Leu 5 Glu Asn Ala Phe 113 Leu 5 | Phe Gly Phe Arg Thr 1111 Val Gly Leu | Arg Gln Lys Ser 110 Pro Ser Arg | Asp His Pro 108 Gly Pro Ala Gln Leu 116 | Glu Gln 107 Ser Gln Ser Gly Arg 115 Asp 5 | Leu 1059 Gly Pro Asp Ala Val 113 Asp O | Glu Val Gly Leu Glu 1120 Asp Pro |

| | Asp | Thr | Leu | Arg | Glu 1205 | | Arg | Tyr | Glu | Ser 1210 | | Pro | Val | Lys | His 1215 | |
|---------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 5 | Thr | Asp | Met | Gly 1220 | | Thr | His | Asn | Lys 1225 | | Leu | Glu | Ala | Asn 1230 | | Asp |
| 10 | Ala | Val | Lys 1235 | | Phe | Ile | Asn | Ala 1240 | | Lys | Lys | Glu | His 1245 | | Gly | Val |
| 10 | Asn | Leu 1250 | | Thr | Arg | Thr | Val 1255 | | Glu | Ser | Gln | Gly 1260 | Ser | Ala | Glu | Leu |
| 15 | Ala 1265 | - | Lys | Leu | Lys | Asn 1270 | | Leu | Leu | Ser | Leu 1279 | | Ser | Gly | Glu | Ser 1280 |
| | Met | Ser | Phe | Ser | Arg 1285 | | Tyr | Gly | Gly | Gly 1290 | | Ser | Thr | Val | Phe 1295 | |
| 20 | Pro | Thr | Leu | Ser 1300 | - | Lys | Val | Pro | Val 1305 | | Val | Ile | Pro | Gly 1310 | | Gly |
| 25 | Ile | Thr | Leu 1315 | _ | Arg | Ala | Tyr | Asn 1320 | | Ser | Phe | Ser | Arg 1325 | | Ser | Gly |
| 23 | Gly | Leu 1330 | | Val | Ser | Phe | Gly 1335 | | Asp | Gly | Gly | Val 1340 | Ser | Gly | Asn | Ile |
| 30 | Met 1345 | | Ala | Thr | Gly | His 1350 | | Val | Met | Pro | Tyr 1359 | | Thr | Gly | Lys | Lys 1360 |
| | Thr | Ser | Ala | Gly | Asn 1365 | | Ser | Asp | Trp | Leu 1370 | | Ala | Lys | His | Lys 137 | |
| 35 | Ser | Pro | Asp | Léu 1380 | | Ile | Gly | Ala | Ala 1385 | | Ser | Gly | Thr | Leu 1390 | | Gly |
| 40 | Thr | Leu | Gln 1395 | | Ser | Leu | Lys | Phe 1400 | | Leu | Thr | Glu | Asp 1405 | | Leu | Pro |
| 10 | Gly | Phe 1410 | | His | Gly | Leu | Thr 1419 | | Gly | Thr | Leu | Thr 1420 | Pro | Ala | Glu | Leu |
| 45 | Leu 1429 | | Lys | Gly | Ile | Glu 1430 | | Gln | Met | Lys | Gln 143 | | Ser | Lys | Leu | Thr 144 |
| · · · · · · · · · · · · · · · · · · · | Phe | Ser | Val | Asp | Thr 1445 | | Ala | Asn | Leu | Asp 145 | | Arg | Ala | Gly | Ile 145 | |
| 50 | Leu | Asn | Glu | Asp 1460 | | Ser | Lys | Pro | Asn 1469 | | Val | Thr | Ala | Arg 1470 | | Ser |
| 55 | Ala | Gly | Leu 1479 | | Ala | Ser | Ala | Asn 1480 | | Ala | Ala | Gly | Ser 1485 | | Glu | Arg |
| | Ser | Thr 1490 | | Ser | Gly | Gln | Phe 149 | | Ser | Thr | Thr | Ser 1500 | Ala O | Ser | Asn | Asn |
| 60 | Arg 1505 | | Thr | Phe | Leu | Asn 1510 | | Val | Gly | Ala | Gly 1519 | | Asn | Leu | Thr | Ala 152 |
| | Ala | Leu | Gly | Val | Ala 1525 | | Ser | Ser | Thr | His 1530 | | Gly | Lys | Pro | Val 1539 | |

| | Ile | Phe | Pro | Ala 1540 | | Thr | Ser | Thr | Asn 1545 | | Ser | Ala | Ala | Leu 1550 | | Leu |
|----|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| 5 | Asp | Asn | Arg 1555 | | Ser | Gln | Ser | 11e 1560 | | Leu | Glu | Leu | Lys 1565 | _ | Ala | Glu |
| | Pro | Val 1570 | | Ser | Asn | Asp | Ile 1579 | | Glu | Leu | Thr | Ser 1580 | | Leu | Gly | Lys |
| 10 | His 1585 | | Lys | Asp | Ser | Ala 1590 | | Thr | Lys | Met | Leu 1595 | | Ala | Leu | Lys | Glu 1600 |
| 15 | Leu | Asp | Asp | Ala | Lys 1605 | Pro | Ala | Glu | Gln | Leu 1610 | | Ile | Leu | Gln | Gln 1615 | |
| 15 | Phe | Ser | Ala | Lys 1620 | _ | Val | Val | Gly | Asp 1625 | | Arg | Tyr | Glu | Ala 1630 | | Arg |
| 20 | Asn | Leu | Lys 1635 | - | Leu | Val | Ile | Arg 1640 | | Gln | Ala | Ala | Asp 1645 | | His | Ser |
| | Met | Glu 1650 | | Gly | Ser | Ala | Ser 1655 | | Ser | Thr | Thr | Tyr 1660 | | Asn | Leu | Ser |
| 25 | Arg 1665 | | Asn. | Asn | Asp | Gly 1670 | | Val | Glu | Leu | Leu 1679 | | Lys | His | Phe | Asp 1680 |
| 30 | Ala | Ala | Leu | Pro | Ala 1689 | Ser | Ser | Ala | Lys | Arg 1690 | | Gly | Glu | Met | Met 1695 | |
| 30 | Asn | Asp | Pro | Ala 1700 | | Lys | Asp | Ile | Ile 1705 | | Gln | Leu | Gln | Ser 1710 | | Pro |
| 35 | Phe | Ser | Ser 1715 | | Ser | Val | Ser | Met 1720 | | Leu | Ĺys | Asp | Gly 1725 | | Arg | Glu |
| | Gln | Thr 1730 | | Lys | Ala | Ile | Leu 1735 | | Gly | Lys | Val | Gly 1740 | | Glu | Glu | Val |
| 40 | Gly 174 | | Leu | Phe | Gln | Asp 1750 | _ | Asn | Asn | Leu | Arg 175 | | Lys | Ser | Val | Ser 1760 |
| 45 | Val | Ser | Gln | Ser | Val 1769 | | Lys | Ser | Glu | Gly 1770 | | Asn | Thr | Pro | Ala 1779 | Leu 5 |
| 75 | Leu | Leu | | Thr 1780 | | Asn | | | | | | | | | | Ile |
| 50 | Gly | Thr | Ile 1799 | | Phe | Lys | Tyr | Gly 1800 | | Asp | Gln | Asn | Thr 180 | | Arg | Arg |
| | Phe | Thr 1810 | | Glu | Gly | Gly | Ile 1819 | | Gln | Ala | Asn | Pro 182 | | Val | Ala | Ser |
| 55 | Ala 182 | | Thr | Asp | Leu | Lys 1830 | | Glu | Gly | Leu | Glu 183 | | Lys | Ser | | |

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 9 as follows:

| | ATGACATCGT | CACAGCAGCG | GGTTGAAAGG | TTTTTACAGT | ATTTCTCCGC | CGGGTGTAAA | 60 |
|----|------------|------------|------------|------------|------------|------------|-----|
| _ | ACGCCCATAC | ATCTGAAAGA | CGGGGTGTGC | GCCCTGTATA | ACGAACAAGA | TGAGGAGGCG | 120 |
| 5 | GCGGTGCTGG | AAGTACCGCA | ACACAGCGAC | AGCCTGTTAC | TACACTGCCG | AATCATTGAG | 180 |
| | GCTGACCCAC | AAACTTCAAT | AACCCTGTAT | TCGATGCTAT | TACAGCTGAA | TTTTGAAATG | 240 |
| 10 | GCGGCCATGC | GCGGCTGTTG | GCTGGCGCTG | GATGAACTGC | ACAACGTGCG | TTTATGTTTT | 300 |
| | CAGCAGTCGC | TGGAGCATCT | GGATGAAGCA | AGTTTTAGCG | ATATCGTTAG | CGGCTTCATC | 360 |
| | GAACATGCGG | CAGAAGTGCG | TGAGTATATA | GCGCAATTAG | ACGAGAGTAG | CGCGGCATAA | 420 |
| 15 | | | | | | | |

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 10 as follows:

| 20 | | | | | | | | | | | | | | | | |
|----------------|-----------|------------|------------|------------|-----------|-----------|------------|------------|------------|-----------|-----------|-----------|------------|------------|-----------|-------------|
| 20 | Met 1 | Thr | Ser | Ser | Gln 5 | Gln | Arg | Val | Glu | Arg 10 | Phe | Leu | Gln | Tyr | Phe 15 | Ser |
| 25 | Ala | Gly | Cys | Lys 20 | Thr | Pro | Ile | His | Leu 25 | Lys | Asp | Gly | Val | Cys 30 | Ala | Leu |
| | Tyr | Asn | Glu 35 | Gln | Asp | Glu | Glu | Ala 40 | Ala | Val | Leu | Glu | Val 45 | Pro | Gln | His |
| 30 | Ser | Asp 50 | Ser | Leu | Leu | Leu | His 55 | Cys | Arg | Ile | Ile | Glu 60 | Ala | Asp | Pro | Gln |
| | Thr 65 | Ser | Ile | Thr | Leu | Tyr 70 | Ser | Met | Leu | Leu | Gln 75 | Leu | Asn | Phe | Glu | Met 80 |
| 35 | Ala | Ala | Met | Arg | Gly 85 | Cys | Trp | Leu | Ala | Leu 90 | Asp | Glu | Leu | His | Asn 95 | Val |
| 40 | Arg | Leu | Cys | Phe 100 | Gln | Gln | Ser | Leu | Glu 105 | His | Leu | Asp | Glu | Ala 110 | Ser | Phe |
| -· - · · · · · | Ser- | Asp | Ile 115 | Val | Ser | _Gly | Phe | Ile 120 | _Glu | His | Ala | Ala | Glu 125 | Val | Arg | Gl u |
| 45 | Tyr | Ile 130 | Ala | Gln | Leu | Asp | Glu 135 | Ser | Ser | Ala | Ala | | | | ٠ | |

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
55 1 5 10 15

| | Ala | Leu | Val | Leu 20 | Val | Arg | Pro | Glu | Ala 25 | Glu | Thr | Thr | Gly | Ser 30 | Thr | Ser |
|----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 5 | Ser | Lys | Ala 35 | Leu | Gln | Glu | Val | Val 40 | Val | Lys | Leu | Ala | Glu 45 | Glu | Leu | Met |
| | Arg | Asn 50 | Gly | Gln | Leu | Asp | Asp 55 | Ser | Ser | Pro | Leu | Gly 60 | Lys | Leu | Leu | Ala |
| | Lys 65 | Ser | Met | Ala | Ala | Asp 70 | Gly | Lys | Ala | Gly | Gly 75 | Gly | Ile | Glu | Asp | Val 80 |
| 10 | Ile | Ala | Ala | Leu | Asp 85 | Lys | Leu | Ile | His | Glu 90 | Lys | Leu | Gly | Asp | Asn 95 | Phe |
| | Gly | Ala | Ser | Ala 100 | Asp | Ser | Ala | Ser | Gly 105 | Thr | Gly | Gln | Gln | Asp 110 | Leu | Met |
| 15 | Thr | Gln | Val 115 | Leu | Asn | Gly | Leu | Ala 120 | Lys | Ser | Met | Leu | Asp 125 | Asp | Leu | Leu |
| | Thr | Lys 130 | Gln | Asp | Gly | Gly | Thr 135 | Ser | Phe | Ser | Glu | Asp 140 | Asp | Met | Pro | Met |
| | Leu 145 | Asn | Lys | Ile | Ala | Gln 150 | Phe | Met | Asp | Asp | Asn 155 | Pro | Ala | Gln | Phe | Pro 160 |
| 20 | Lys | Pro | Asp | Ser | Gly 165 | Ser | Trp | Val | Asn | Glu 170 | Leu | Lys | Glu | Asp | Asn 175 | |
| | Leu | Asp | Gly | Asp 180 | Glu | Thr | Ala | Ala | Phe 185 | Arg | Ser | Ala | Leu | Asp 190 | Ile | Ile |
| 25 | Gly | Gln | Gln 195 | Leu | Gly | Asn | Gln | Gln 200 | Ser | Asp | Ala | Gly | Ser 205 | Leu | Ala | Gly |
| | Thr | Gly 210 | Gly | Gly | Leu | Gly | Thr 215 | Pro | Ser | Ser | Phe | Ser 220 | Asn | Asn | Ser | Ser |
| | Val 225 | Met | Gly | Asp | Pro | Leu 230 | Ile | Asp | Ala | Asn | Thr 235 | Gly | Pro | Gly | Asp | Ser 240 |
| 30 | Gly | Asn | Thr | Arg | Gly 245 | Glu | Ala | Gly | Gln | Leu 250 | Ile | Gly | Glu | Leu | 11e 255 | Asp |
| | Arg | Gly | Leu | Gln 260 | Ser | Val | Leu | Ala | Gly 265 | Gly | Gly | Leu | Gly | Thr 270 | Pro | Val |
| 35 | Asn | Thr | Pro 275 | Gln | Thr | Gly | Thr | Ser 280 | Ala | Asn | Gly | Gly | Gln 285 | Ser | Ala | Gln |
| | Asp | Leu 290 | Asp | Gln | Leu | Leu | Gly 295 | Gly | Leu | Leu | Leu | Lys 300 | Gly | Leu | Glu | Ala |
| | Thr 305 | Leu | Lys | Asp | Ala | Gly 310 | Gln | Thr | Gly | Thr | Asp 315 | Val | Gln | Ser | Ser | Ala 320 |

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg

Asn Gln Ala Ala Ala

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This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from Pseudomonas syringae is found in He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas syringae pv. syringae Harpiness: a Protein that is Secreted via the Hrp 10 Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from Pseudomonas syringae has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCCTG 20

GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC 120 GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA 180 AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC 240 ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG 300 360 AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC 420 GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC 480 AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC 540 GAAACGGCTG CGTTCCGTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG 600 AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC 660 AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC 720 GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA 780 TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCGCAGAC CGGTACGTCG 840 GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG 900 GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT 960 GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA 1020 1026 **GCCTGA**

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 13 as follows:

| | TCCACTTCGC | TGATTTTGAA | ATTGGCAGAT | TCATAGAAAC | GTTCAGGTGT | GGAAATCAGG | 60 |
|----|------------|------------|------------|------------|------------|------------|------|
| 10 | CTGAGTGCGC | AGATTTCGTT | GATAAGGGTG | TGGTACTGGT | CATTGTTGGT | CATTTCAAGG | 120 |
| 10 | CCTCTGAGTG | CGGTGCGGAG | CAATACCAGT | CTTCCTGCTG | GCGTGTGCAC | ACTGAGTCGC | 180 |
| | AGGCATAGGC | ATTTCAGTTC | CTTGCGTTGG | TTGGGCATAT | AAAAAAAGGA | ACTTTTAAAA | 240 |
| 15 | ACAGTGCAAT | GAGATGCCGG | CAAAACGGGA | ACCGGTCGCT | GCGCTTTGCC | ACTCACTTCG | 300 |
| | AGCAAGCTCA | ACCCCAAACA | TCCACATCCC | TATCGAACGG | ACAGCGATAC | GGCCACTTGC | 360 |
| 20 | TCTGGTAAAC | CCTGGAGCTG | GCGTCGGTCC | AATTGCCCAC | TTAGCGAGGT | AACGCAGCAT | 420 |
| 20 | GAGCATCGGC | ATCACACCCC | GGCCGCAACA | GACCACCACG | CCACTCGATT | TTTCGGCGCT | 480 |
| | AAGCGGCAAG | AGTCCTCAAC | CAAACACGTT | CGGCGAGCAG | AACACTCAGC | AAGCGATCGA | 540 |
| 25 | CCCGAGTGCA | CTGTTGTTCG | GCAGCGACAC | ACAGAAAGAC | GTCAACTTCG | GCACGCCCGA | 600 |
| | CAGCACCGTC | CAGAATCCGC | AGGACGCCAG | CAAGCCCAAC | GACAGCCAGT | CCAACATCGC | 660 |
| 30 | TAAATTGATC | AGTGCATTGA | TCATGTCGTT | GCTGCAGATG | CTCACCAACT | CCAATAAAAA | 720 |
| 30 | GCAGGACACC | AATCAGGAAC | AGCCTGATAG | CCAGGCTCCT | TTCCAGAACA | ACGGCGGCT | 780 |
| | CGGTACACCG | TCGGCCGATA | GCGGGGGCGG | CGGTACACCG | GATGCGACAG | GTGGCGGCGG | 840 |
| 35 | CGGTGATACG | CCAAGCGCAA | CAGGCGGTGG | CGGCGGTGAT | ACTCCGACCG | CAACAGGCGG | 900 |
| | TGGCGGCAGC | GGTGGCGGCG | GCACACCCAC | TGCAACAGGT | GGCGGCAGCG | GTGGCACACC | 960 |
| 40 | CACTGCAACA | GGCGGTGGCG | AGGGTGGCGT | AACACCGCAA | ATCACTCCGC | AGTTGGCCAA | 1020 |
| | CCCTAACCGT | ACCTCAGGTA | CTGGCTCGGT | GTCGGACACC | GCAGGTTCTA | CCGAGCAAGC | 1080 |
| | CGGCAAGATC | AATGTGGTGA | AAGACACCAT | CAAGGTCGGC | GCTGGCGAAG | TCTTTGACGG | 1140 |
| 45 | CCACGGCGCA | ACCTTCACTG | CCGACAAATC | TATGGGTAAC | GGAGACCAGG | GCGAAAATCA | 1200 |
| • | GAAGCCCATG | TTCGAGCTGG | CTGAAGGCGC | TACGTTGAAG | AATGTGAACC | TGGGTGAGAA | 1260 |
| 50 | CGAGGTCGAT | GGCATCCACG | TGAAAGCCAA | AAACGCTCAG | GAAGTCACCA | TTGACAACGT | 1320 |
| | GCATGCCCAG | AACGTCGGTG | AAGACCTGAT | TACGGTCAAA | GGCGAGGGAG | GCGCAGCGGT | 1380 |
| | CACTAATCTG | AACATCAAGA | ACAGCAGTGC | CAAAGGTGCA | GACGACAAGG | TTGTCCAGCT | 1440 |
| 55 | CAACGCCAAC | ACTCACTTGA | AAATCGACAA | CTTCAAGGCC | GACGATTTCG | GCACGATGGT | 1500 |
| | TCGCACCAAC | GGTGGCAAGC | AGTTTGATGA | CATGAGCATC | GAGCTGAACG | GCATCGAAGC | 1560 |
| 60 | TAACCACGGC | AAGTTCGCCC | TGGTGAAAAG | CGACAGTGAC | GATCTGAAGC | TGGCAACGGG | 1620 |

WO 00/28055 PCT/US99/26039

- 25 -

CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680
CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC 1729

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This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 14 as follows:

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| 10 | Met 1 | Ser | Ile | Gly | Ile 5 | Thr | Pro | Arg | Pro | Gln 10 | Gln | Thr | Thr | Thr | Pro 15 | Leu |
|----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 15 | Asp | Phe | Ser | Ala 20 | Leu | Ser | Gly | Lys | Ser 25 | Pro | Gln | Pro | Asn | Thr 30 | Phe | Gly |
| | Glu | Gln | Asn 35 | Thr | Gln | Gln | Ala | Ile 40 | Asp | Pro | Ser | Ala | Leu 45 | Leu | Phe | Gly |
| 20 | Ser | Asp 50 | Thr | Gln | Lys | Asp | Val 55 | Asn | Phe | Gly | Thr | Pro 60 | Asp | Ser | Thr | Val |
| 25 | Gln 65 | Asn | Pro | Gln | Asp | Ala 70 | Ser | Lys | Pro | Asn | Asp 75 | Ser | Gln | Ser | Asn | Ile 80 |
| 23 | Ala | Lys | Leu | Ile | Ser 85 | Ala | Leu | Ile | Met | Ser 90 | Leu | Leu | Gln | Met | Leu 95 | Thr |
| 30 | Asn | Ser | Asn | Lys 100 | Lys | Gln | Asp | Thr | Asn 105 | Gln | Glu | Gln | Pro | Asp 110 | | Gln |
| | Ala | Pro | Phe 115 | Gln | Asn | Asn | Gly | Gly 120 | Leu | Gly | Thr | Pro | Ser 125 | Ala | Asp | Ser |
| 35 | Gly | Gly 130 | Gly | Gly | Thr | Pro | Asp 135 | Ala | Thr | Gly | Gly | Gly 140 | Gly | Gly | Asp | Thr |
| 40 | Pro 145 | Ser | Ala | Thr | Gly | Gly 150 | Gly | Gly | Gly | Asp | Thr 155 | Pro | Thr | Ala | Thr | Gly 160 |
| 40 | Gly | Gly | Gly | Ser | Gly 165 | Gly | Gly | Gly | Thr | Pro 170 | Thr | Ala | Thr | Gly | Gly 175 | Gly |
| 45 | Ser | Gly | Gly | Thr 180 | Pro | Thr | Ala | Thr | Gly 185 | Gly | Gly | Glu | Gly | Gly 190 | | Thr |
| | Pro | Gln | Ile 195 | Thr | Pro | Gln | Leu | Ala 200 | Asn | Pro | Asn | Arg | Thr 205 | Ser | Gly | Thr |
| 50 | Gly | Ser 210 | Val | Ser | Asp | Thr | Ala 215 | Gly | Ser | Thr | Glu | Gln 220 | Ala | Gly | Lys | Ile |
| 55 | Asn 225 | Val | Val | Lys | Asp | Thr 230 | Ile | Lys | Val | Gly | Ala 235 | Gly | Glu | Val | Phe | Asp 240 |

| | Gly | His | Gly | Ala | Thr 245 | Phe | Thr | Ala | Asp | Lys 250 | Ser | Met | Gly | Asn | Gly 255 | Asp |
|----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 5 | Gln | Gly | Glu | Asn 260 | Gln | Lys | Pro | Met | Phe 265 | Glu | Leu | Ala | Glu | Gly 270 | Ala | Thr |
| | Leu | Lys | Asn 275 | Val | Asn | Leu | Gly | Glu 280 | Asn | Glu | Val | Asp | Gly 285 | Ile | His | Val |
| 10 | Lys | Ala 290 | Lys | Asn | Ala | Gln | Glu 295 | Val | Thr | Ile | Asp | Asn 300 | Val | His | Ala | Gln |
| 15 | Asn 305 | Val | Gly | Glu | Asp | Leu 310 | Ile | Thr | Val | Lys | Gly 315 | Glu | Gly | Gly | Ala | Ala 320 |
| | Val | Thr | Asn | Leu | Asn 325 | Ile | Lys | Asn | Ser | Ser 330 | Ala | Lys | Gly | Ala | Asp 335 | Asp |
| 20 | Lys | Val | Val | Gln 340 | Leu | Asn | Ala | Asn | Thr 345 | His | Leu | Lys | Ile | Asp 350 | Asn | Phe |
| | Lys | Ala | Asp 355 | Asp | Phe | Gly | Thr | Met 360 | Val | Arg | Thr | Asn | Gly 365 | Gly | Lys | Gln |
| 25 | Phe | Asp 370 | Asp | Met | Ser | Ile | Glu 375 | Leu | Asn | Gly | Ile | Glu 380 | Ala | Asn | His | Gly |
| 30 | Lys 385 | Phe | Ala | Leu | Val | Lys 390 | Ser | Asp | Ser | Asp | Asp 395 | Leu | Lys | Leu | Ala | Thr 400 |
| , | Gly | Asn | Ile | Ala | Met 405 | Thr | Asp | Val | Lys | His 410 | Ala | Tyr | Asp | Lys | Thr 415 | Gln |
| 35 | Ala | Ser | Thr | Gln 420 | His | Thr | Glu | Leu | | | | | | | | |

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 15 as follows:

| 45 | Met 1 | Ser | Val | Gly | Asn 5 | Île | Gln | Ser | | Ser 10 | Asn | Leu | Pro | Gly | Leu 15 | Gln |
|----|----------|-----------|-----------|-----------|----------|-----|-----------|-----------|-----------|-----------|-----|-----------|-----------|-----------|-----------|-----|
| | Asn | Leu | Asn | Leu 20 | Asn | Thr | Asn | Thr | Asn 25 | Ser | Gln | Gln | Ser | Gly 30 | Gln | Ser |
| | Val | Gln | Asp 35 | Leu | Ile | Lys | Gln | Val 40 | Glu | Lys | Asp | Ile | Leu 45 | Asn | Ile | Ile |
| 50 | Ala | Ala 50 | Leu | Val | Gln | Lys | Ala 55 | Ala | Gln | Ser | Ala | Gly 60 | Gly | Asn | Thr | Gly |

| | Asn 65 | Thr | Gly | Asn | Ala | Pro 70 | Ala | Lys | Asp | Gly | Asn 75 | Ala | Asn | Ala | Gly | Ala 80 |
|----|------------|------------|-------------|------------|------------|------------|------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|
| | Asn | Asp | Pro | Ser | Lys 85 | Asn | Asp | Pro | Ser | Lys 90 | Ser | Gln | Ala | Pro | Gln 95 | Ser |
| 5 | Ala | Asn | Lys | Thr 100 | Gly | Asn | Val | Asp | Asp 105 | Ala | Asn | Asn | Gln | Asp 110 | Pro | Met |
| | Gln | Ala | Leu 115 | Met | Gln | Leu | Leu | Glu 120 | Asp | Leu | Val | Lys | Leu 125 | Leu | Lys | Ala |
| 10 | Ala | Leu 130 | His | Met | Gln | Gln | Pro 135 | Gly | Gly | Asn | Asp | Lys 140 | Gly | Asn | Gly | Val |
| | Gly 145 | Gly | Ala | Asn | Gly | Ala 150 | Lys | Gly | Ala | Gly | Gly 155 | Gln | Gly | Gly | Leu | Ala 160 |
| | Glu | Ala | Leu | Gln | Glu 165 | Ile | Glu | Gln | Ile | Leu 170 | Ala | Gln | Leu | Gly | Gly 175 | Gly |
| 15 | Gly | Ala | Gly | Ala 180 | Gly | Gly | Ala | Gly | Gly 185 | Gly | Val | Gly | Gly | Ala 190 | Gly | Gly |
| | Ala | Asp | Gly 195 | Gly | Ser | Gly | Ala | Gly 200 | Gly | Ala | Gly | Gly | Ala 205 | Asn | Gly | Ala |
| 20 | Asp | Gly 210 | Gly | Asn | Gly | Val | Asn 215 | Gly | Asn | Gln | Ala | Asn 220 | Gly | Pro | Gln | Asn |
| | Ala 225 | Gly | Asp | Val | Asn | Gly 230 | Ala | Asn | Gly | Ala | Asp 235 | Asp | Gly | Ser | Glu | Asp 240 |
| | Gln | Gly | Gly | Leu | Thr 245 | Gly | Val | Leu | Gln | Lys 250 | Leu | Met | Lys | Ile | Leu 255 | Asn |
| 25 | Ala | Leu | Val | Gln 260 | Met | Met | Gln | Gln | Gly 265 | Gly | Leu | Gly | Gly | Gly 270 | Asn | Gln |
| | Ala | Gln | Gly -275 | Gly | Ser | Lys | Gly | Ala -280 | Gly | Asn | Ala | Ser | Pro 285 | Ala | Ser | Gly |
| 30 | Ala | Asn 290 | Pro | Gly | Ala | Asn | Gln 295 | Pro | Gly | Ser | Ala | Asp 300 | Asp | Gln | Ser | Ser |
| | Gly 305 | Gln | Asn | Asn | Leu | Gln 310 | Ser | Gln | Ile | Met | Asp 315 | Val | Val | Lys | Glu | Val 320 |
| | Val | Gln | Ile | Leu | Gln 325 | Gln | Met | Leu | Ala | Ala 330 | Gln | Asn | Gly | Gly | Ser 335 | Gln |
| 35 | Gln | Ser | Thr | Ser 340 | Thr | Gln | Pro | Met | | | | | | | | |

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 16 as follows:

| | ATGTCAGTCG | GAAACATCCA | GAGCCCGTCG | AACCTCCCGG | GTCTGCAGAA | CCTGAACCTC | 60 |
|----|------------|-----------------|------------|------------|------------|------------|------|
| | AACACCAACA | CCAACAGCCA | GCAATCGGGC | CAGTCCGTGC | AAGACCTGAT | CAAGCAGGTC | 120 |
| | GAGAAGGACA | TCCTCAACAT | CATCGCAGCC | CTCGTGCAGA | AGGCCGCACA | GTCGGCGGGC | 180 |
| | GGCAACACCG | GTAACACCGG | CAACGCGCCG | GCGAAGGACG | GCAATGCCAA | CGCGGGCGCC | 240 |
| 5 | AACGACCCGA | GCAAGAACGA | CCCGAGCAAG | AGCCAGGCTC | CGCAGTCGGC | CAACAAGACC | 300 |
| | GGCAACGTCG | ACGACGCCAA | CAACCAGGAT | CCGATGCAAG | CGCTGATGCA | GCTGCTGGAA | 360 |
| | GACCTGGTGA | AGCTGCTGAA | GGCGGCCCTG | CACATGCAGC | AGCCCGGCGG | CAATGACAAG | 420 |
| | GGCAACGGCG | TGGGCGGTGC | CAACGGCGCC | AAGGGTGCCG | GCGGCCAGGG | CGGCCTGGCC | 480 |
| | GAAGCGCTGC | AGGAGATCGA | GCAGATCCTC | GCCCAGCTCG | GCGGCGGCGG | TGCTGGCGCC | 540 |
| 10 | GGCGGCGCGG | GTGGCGGTGT | CGGCGGTGCT | GGTGGCGCGG | ATGGCGGCTC | CGGTGCGGGT | 600 |
| | GGCGCAGGCG | GTGCGAACGG | CGCCGACGGC | GGCAATGGCG | TGAACGGCAA | CCAGGCGAAC | 660 |
| | GGCCCGCAGA | ACGCAGGCGA | TGTCAACGGT | GCCAACGGCG | CGGATGACGG | CAGCGAAGAC | 720 |
| | CAGGGCGGCC | TCACCGGCGT | GCTGCAAAAG | CTGATGAAGA | TCCTGAACGC | GCTGGTGCAG | 780 |
| | ATGATGCAGC | AAGGCGGCCT | ceeceeceec | AACCAGGCGC | AGGGCGGCTC | GAAGGGTGCC | 840 |
| 15 | GGCAACGCCT | CGCCGGCTTC | CGGCGCGAAC | CCGGGCGCGA | ACCAGCCCGG | TTCGGCGGAT | 900 |
| | GATCAATCGT | CCGGCCAGAA | CAATCTGCAA | TCCCAGATCA | TGGATGTGGT | GAAGGAGGTC | 960 |
| | GTCCAGATCC | TGCAGCAGAT | GCTGGCGGCG | CAGAACGGCG | GCAGCCAGCA | GTCCACCTCG | 1020 |
| | ACCCACCCGA | דכיד א א | | | | | 1035 |

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Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," <u>EMBO J.</u> 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

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Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala 1 5 10 15

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Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
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This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from

Xanthomonas campestris pv. pelargonii is heat stable, protease sensitive, and has a
molecular weight of 20 kDa. It includes an amino acid sequence corresponding to

SEQ. ID. No. 18 as follows:

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln

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Leu Leu Ala Met
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Isolation of Erwinia carotovora hypersensitive response elictor protein or polypeptide is described in Cui et al., "The RsmA Mutants of Erwinia carotovora subsp. carotovora Strain Ecc71 Overexpress hrp N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of Erwinia stewartii is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of Erwinia stewartii on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of Erwinia stewartii on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamoni, Phytophthora capsici, Phytophthora megasperma, and Phytophora citrophthora are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and

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Acquired Resistance in Tobacco," <u>Eur. J. Biochem.</u>, 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," <u>Plant Path.</u> 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," <u>Plant J.</u>, 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," <u>Eur. J. Plant Path.</u>, 102:181-92 (1996), which are hereby incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. sepedonicus which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

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In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia*. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of suitable fragments of a hypersensitive response elicitor which do elicit a hypersensitive response are *Erwinia amylovora* fragments including a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the

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following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.

Suitable DNA molecules are those that hybridize to the DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 2, 4, 5, 7, 9, 12, 13, and 16 under stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 µm g/ml *E. coli* DNA.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which cotranslationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the hypersensitive response elicitor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the hypersensitive response elicitor is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an

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expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the proteinencoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;

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microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promotor, *lac* promotor,

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trp promotor, recA promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to lacUV5, ompF, bla, lpp, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid trp-lacUV5 (tac) promotor or other E. coli promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

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The present invention's method of imparting stress resistance to plants can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the elicitor to impart stress resistance. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart stress resistance in plants.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart stress resistance in plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart stress resistance to plants. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart stress resistance to plants.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated hypersensitive response elicitor or 2) application of bacteria which do not cause disease and are transformed with a genes encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding a hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seed cells. In these embodiments, the elicitor is produced by the bacteria in planta or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea,

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chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

In accordance with the present invention, the term "stress" refers to drought, salt, cold temperatures (e.g., frost), chemical treatment (e.g., insecticides, fungicides, herbicides, fertilizers), water, excessive light, and insufficient light.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants.

The hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

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polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM of the elicitor.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include (NH₄)₂NO₃. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby

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incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., <u>Proc. Natl. Acad. Sci. USA</u>, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., <u>Proc. Natl. Acad. Sci. USA</u>, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with Agrobacterium tumefaciens or A. rhizogenes previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (A. tumefaciens) and hairy root disease (A. rhizogenes). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a

convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of A. tumefaciens or the Ri plasmid of A. rhizogenes. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

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After transformation, the transformed plant cells must be regenerated. Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures. Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the

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gene encoding the hypersensitive response elicitor resulting in stress resistance to the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart stress resistance to plants. While not wishing to be bound by theory, such stress resistance may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor in accordance with the present invention to impart stress resistance. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

<u>Example 1</u> - Hypersensitive Response Elicitor-Treated Cotton is More Resistant to the Damage Caused by Insecticide Stress

Aphids (Aphids gossypii) infect cotton during the entire growth season. The damage of aphid infection ranges from honeydew deposit that contaminates the lint and reduces crop value to defoliation that reduces or destroys crops. To protect plants from aphid infection, cotton is usually sprayed with insecticides, for example Asana XL when the infection pressure is not very high, and Admire when the infestation pressure is high. The effect of a hypersensitive response elicitor on aphids in cotton was studied by a trial involving a randomized complete block design. This

involved treatment with *Erwinia amylovora* hypersensitive response elicitor (i.e. HP-1000™) at 20, 60, and 80 ppm and a chemical insecticide, Asana XL, at 8 oz./ac. Each treatment involved foliar application beginning at cotyledon to three true leaves and thereafter at 14 day intervals using a backpack sprayer. Aphid counts and overall growth of the cotton were made immediately prior to spray application at 14, 28, 35, and 42 days after the first treatment ("DAT 1"). Twenty-five randomly selected leaves per plot were collected at the first three sampling dates and the leaves per plot at the final sampling date.

10 Results

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1. Aphid control: The number of aphids in the hypersensitive response elicitor-treated cotton were significantly reduced in comparison to the chemical treated cotton (see Table 1).

Table 1. Aphid count per leaf on cotton after treatment with Asana XL[®] or HP-1000[™]

Number of aphids per leaf No. sprays applied/days after treatment Rate² 1/14DAT1 2/28DAT1 3/35DAT1 4/42DAT1 Treatment Asana XL 8 oz/ac 0.2 a 32.2 a 110.0 a 546.9 a HP-1000™ 0.2 a 7.8 b 22.9 b 20 μg/ml 322.1 a HP-1000™ 60 μg/ml 0.1 a 4.9 b 34.6 b 168.3 a 80 µg/ml 0.0 a 2.7 b 25.8 b 510.2 a HP-1000™

^TMeans followed by different letters are significantly different according to Duncan's MRT, P=0.05. ²Rate for Asana XL[®] is for formulated product, rate for HP-1000™ is for active ingredient (a.i.).

At 14 days after DAT 1, aphid counts were relatively low across all of

the treatments, but by 28 days after DAT 1 (by which time two sprayings had been applied), the number of aphids per leaf were significantly greater in Asana XL-treated plants compared to the hypersensitive response elicitor-treated cottons. By 35 days after DAT 1 (by which time three sprayings had been applied), aphid counts had risen for all treatments, yet aphid counts per leaf were still significantly lower for hypersensitive response elicitor-treated cotton compared to the Asana XL treatment.

Finally, at 42 days after DAT 1 (by which time four sprayings had been applied), the

Finally, at 42 days after DAT 1 (by which time four sprayings had been applied), the number of aphids per leaf had increased to a level that threatened to overwhelm the

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plants even when treated with the standard chemical insecticide. To save the trial, another chemical, Pravado (Admire), was applied to all plots to eradicate aphids from the field.

2. Hypersensitive response elicitor-treated cotton was more resistant to the damage caused by Pravado (Admire) and Asana. After the second chemical spraying, it was observed that cotton plants were stress shocked by the insecticides. The cotton plants previously treated with Asana and untreated control were defoliated. On most of the chemical-treated cotton, there were no leaves, or very few leaves, in the lower portion of plants. However, the hypersensitive response elicitor-treated plants, especially the plot where hypersensitive response elicitor was applied at 80 ppm, had no defoliation and the cotton plants were vigorous and healthy. By counting the number of mature balls, it clearly showed that hypersensitive response elicitor-treated plants (at 80 ppm) had more ball setting than chemical and untreated control (Table 2), indicating that hypersensitive response elicitor-treated plants were more tolerant to the stress caused by insecticide.

Table 2. Number of Formed Cotton Balls Counted on Ten Plants in Each of Four Replicates Per Treatment.

| 20 | Treatment | No. balls/10 plants/replicate |
|----|----------------------------------|-------------------------------|
| | UTC | 28 |
| | Chemical standard | 6 |
| | Hypersensitive Response Elicitor | 35 |
| 25 | | |

<u>Example 2</u> - Hypersensitive Response Elicitor-Treated Cucumbers are More Resistant to Drought

A cucumber field trial was set up to test the effect of *Erwinia* amylovora hypersensitive response elicitor on disease control, tolerance to drought stress, and yield. Three different rates were tested, there at 15, 30, and 60 µg/ml. In addition to hypersensitive response elicitor treatment, there was an untreated control. Each treatment contained three replicate plots. When the first true leaf emerges, hypersensitive response elicitor was sprayed with a back bag sprayer. The second spray was applied ten days after the first spray. The third application was right after

- 44 -

the recovery of cucumber seedlings after the transplanting to the field. Individual treatment was randomly assigned in the field.

When the first true leaf emerged (Day 0), a first application was sprayed. Usually cucumber seedlings are transplanted when seedlings show two true leaves. It has been known that the recovery rate after the transplanting is closely related to the size of the seedlings. Because of the drought, the seedlings were maintained in the nursery for an extra ten days and the second spray was applied on Day 10. Two days after the second spray, the plants were transplanted into fields and covered with plastic sheets. The plants had 4 – 5 true leaves.

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Result

The recovery rate of the transplanted cucumber seedlings was higher for the hypersensitive response elicitor-treated plants than for the untreated control. More than 80% of the hypersensitive response elicitor-treated cucumber seedlings survived, while only 57% untreated plants survived.

Throughout the growth season, there was a serious drought problem. Early field visits indicated that hypersensitive response elicitor-treated plants had more root mass and better over-all growth. Hypersensitive response elicitor-treated cucumber started to flower 14 days earlier than untreated control cucumber. The early flowering resulted in an earlier harvest. In the first harvest, more than 0.4 kilograms of cucumber fruits per plant were harvested from the hypersensitive response elicitor-treated cucumbers; however, virtually no fruit was harvested from untreated control. By the end of the season, untreated plants died due to severe drought, but hypersensitive response elicitor-treated plants were still alive and had one more harvest.

The final yield was significantly different between hypersensitive response elicitor-treated and untreated plants. Hypersensitive response elicitor administered at the rate of 30 ppm produced three times greater yield than the control plants (Table 3).

PCT/US99/26039

Table 3. Yield Increase of Cucumber Fruit from Hypersensitive Response Elicitor Treated Plants

| Treatment | Replicate | kg/plant | Yield/ | Replicate | % of the Yield Increase |
|-----------|-----------|----------|--------|-----------|----------------------------|
| | l | 1.25 | 37.5 | | |
| HP 15 | II: | 1.00 | 30.0 | 103.8 | 241 |
| | III | 1.21 | 36.3 | | |
| | I | 1.54 | 46.2 | | |
| HP 30 | II | 1.43 | 42.9 | 133.2 | 339 |
| | III | 1.47 | 44.1 | | |
| | I | 0.43 | 12.9 | | |
| Control | H | 0.41 | 12.3 | 39.3 | |
| | III | 0.47 | 14.1 | | |

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The increased yield was partially attributed to hypersensitive response elicitor-induced growth enhancement and partially resulted from more tolerance of hypersensitive response elicitor-treated cucumber to drought, because usually the yield increase from hypersensitive response elicitor-induced growth enhancement is between 10-40%.

Example 3 - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress

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Pepper seedlings were drenched with hypersensitive response elicitor at 20 ppm seven days before transplanting, sprayed seven days after the transplanting, and then, sprayed every fourteen days. Standard chemicals, Brave, Maneb, Kocide, and Admire, were used for the rest of the treatment. In addition to early growth enhancement, which resulted in a higher yield, larger fruit, and resistance to several diseases, hypersensitive response elicitor-treated pepper was more tolerant to herbicide damage. The pepper field was applied with the herbicide SENCOR which is not labeled for pepper. This herbicide is known to cause severe foliar damage to pepper in chemically-treated plants but not with hypersensitive response elicitor-treated plants.

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The difference between the adverse effect of the herbicide on the hypersensitive response elicitor and non-hypersensitive response elicitor treated plants is dramatic. See Table 4 below. Thirty-nine of the 60 elicitor-treated plants showed only minor damage by the herbicide, the damaged leaves were less than 20%. In

PCT/US99/26039

contrast, 53 out of the 60 chemically-treated pepper plants had severe damage, 40-57% of the leaves were damaged, and 20 plants were dead. The ability of hypersensitive response elicitors to help crops withstand the phytotoxic effects of a herbicide is very important benefit to in agricultural industry.

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Table 4. Hypersensitive Response Elicitor-Treated Peppers are More Tolerant to Herbicide Damage.

| 10 | Treatment | <u>Dan</u> | Damage Rating | | | | | Damage Index % |
|----|---------------------|------------|---------------|------|--------|-------|---------|---------------------------|
| 10 | Hypersensitive | 1 | 2 | 3 | 4 | 5 | 6 | 41 |
| | Response Elicitor | 1 | 38 | 17 | 3 | 1 | 0 | |
| 15 | Chemicals 0 | 1 | 6 | 16 | 19 | 18 | | 87 |
| | Damage Rating: 1. N | o dama | ge; 2. C | -20% | leaves | damag | ged; 3. | 20-40% leaves damaged; 4. |

40-50% leaves damaged; 6. More than 75% leaves damaged or entire plant dead.

Damage index = sum of each rating times the number of plants under the rating scale, divided by total number of plants times 6.

Damage index for hypersensitive response elicitor-treated plants = $1 \times 1 + 2 \times 38 + 3 \times 17 + 4 \times 3 + 5 \times 1 + 6 \times 0$ x 100% = 41%

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<u>Example 4</u> - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress under Controlled Experimental Conditions

A field trial was conducted to test if hypersensitive elicitor treated pepper would be more tolerant to herbicide stress. The trial contains 6 treatments and 4 replicates for each treatment. The treatments are described as follows:

- Control, the peppers were neither treated by a hypersensitive
 response ("HR") elicitor nor by LEXONETM herbicide (DuPont Agricultural Products, Wilmington, Delaware).
 - 2. Control pepper with application of 0.15 pound LEXONE™ herbicide /acre.
- 3. Control pepper with application of 0.3 pound LEXONE™ 40 herbicide /acre.

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- 4. HR elicitor treatment with no application of LEXONETM herbicide using a formulated product known as MESSENGERTM biopesticide (Eden Bioscience Corporation, Bothell, Washington) containing 3% HR elicitor protein was used.
- 5. HR elicitor treatment with application of 0.15 pound LEXONETM herbicide /acre.
 - 6. HR elicitor treatment with application of 0.3 pound LEXONE™ herbicide /acre.

LEXONETM contains the same active ingredient as SENCORTM

10 herbicide (Bayer, Kansas City, Missouri) used in Example 3. Pepper seedlings were drenched with MESSENGERTM solution at the concentration of HR elicitor protein of about 20 ppm seven days before transplanting into the field and then sprayed every 14 days after the transplanting. LEXONE was applied at high (0.3 pound/acre) and low levels (0.15 pound/acre). 50 gallon water and 100 mL of the herbicide solution was introduced into the root zone of each plant in the respective treatment five weeks after transplant into the field.

The treatments were evaluated for the percent of chlorosis caused by the LEXONETM herbicide application and for the pepper yield. HR elicitor-treated plants exposed to the high rate of herbicide had significantly less chlorosis and produced 108 % more fruit in comparison to the non-hypersensitive response elicitor treated plants exposed to the same amount of herbicide. See Tables 5 and 6 below. There was no significant difference in the reduction of chlorosis at the low rate of herbicide between the HR elicitor treated and non-HR elicitor treated peppers. However, the HR elicitor treated plants produced 15% more fruit than the corresponding control plants exposed to the same amount of herbicide. There was no chlorosis in either the check or HR elicitor-treated plants that did not receive LEXONETM herbicide treatment.

The HR elicitor treated plants were much less severely affected by the herbicide application than the respective control plants at the high rate of herbicide. However, the amount of visual chlorosis was similar at the low rate for both the check and HR elicitor-treated plants. More importantly, the yields from both the high and low rate herbicide treatments of HR elicitor treated plants were less severely effected

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by the herbicide than the checks. These findings further confirm that HR elicitors can help crops withstand the phytotoxic effects of herbicides and are very beneficial to the agricultural industry.

Table 5. Reduction of Foliar Chlorosis and Increase in Yield in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONE™ Herbicide

| | | Percent foliar chlorosis and yield of pepper | | | | | | | | |
|--|-------|--|-------|-------|-------|------------------|---|--|--|--|
| Treatment | A | В | C | D | E | Yield (pound) | % difference from the respective control | | | |
| 6 (MESSENGERTM+ High rate LEXONETM) | 13.75 | 30.00 | 37.50 | 36.25 | 40.00 | 8.31 | 108 % | | | |
| 3 (High rate LEXONETM) | 26.25 | 43.75 | 51.25 | 50.00 | 51.25 | 4.00 | - | | | |
| 5 (MESSENGER TM + low rate LENOXE TM) | 16.25 | 22.50 | 28.75 | 23.75 | 27.50 | 8.00 | 15 % | | | |
| 2 (LENOXETM) | 12.50 | 20.00 | 25.00 | 25.00 | 23.75 | 6.81 | - | | | |

Table 6. Weight of Harvested Peppers Increased in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONETM Herbicide Compared to Check Plants.

| Treatment | Weight of peppers harvested 12/1/98 in pounds |
|---------------------------|---|
| HP20 + high rate LEXONE™ | 8.31 |
| Check + high rate LEXONE™ | 4.00 |
| HP20 + low rate LEXONE™ | 8.00 |
| Check ÷ low rate LEXONE™ | 6.81 |

15 <u>Example 5</u> - Hypersensitive Response Elicitor-Treated Cotton is More Tolerant to Drought Stress

A non-irrigated cotton trial experienced 26 consecutive days of drought. The average daily heat index was near or over 100 degrees F, adding to the stress placed on the plants in the field.

Observations in the field indicated that plants treated with HR elicitor at the concentration of 15 ppm (2.2 oz formulated product, MESSENGERTM containing 3 % active ingredient HR elicitor protein) were more vigorous and had less defoliation than the check plants as a result of the heat and drought stress. Equal numbers of plants from the MESSENGERTM-treated and the non-MESSENGERTM treated plots were carefully removed from the field and mapped for the number of nodes and bolls by position. The plants were also weighed on a Metler analytical scale to determine whole plant, root and shoot weights.

MESSENGERTM treated plants survived the heat and drought stresses much better than the untreated plants did. Plants treated with MESSENGERTM had 37.6% more root and shoot mass than the check plants (Table 7). The MESSENGERTM treated plants also had significantly more cotton bolls than the check plants (Table 8). The number of cotton bolls from positions 1 and 2 have a significant contribution to the overall yield. Table 8 showed that MESSENGERTM treated plants had 47% more bolls in positions 1 and 2 and 57% more boll from a whole plant in comparison to the yield achieved using a grower standard treatment (i.e. with no MESSENGERTM treatment). A common reaction to stress in cotton is for the plant to abort bolls. The results indicate that MESSENGERTM-treated plants are more tolerant to the drought stress.

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Table 7. Weight per Plant of Non-Irrigated Cotton Following 26 Days of Drought.

| Treatment | Root weight | %Difference | Shoot weight | % | Whole plant | % |
|------------------------------------|--------------|-------------|--------------|------------|---------------------|------------|
| | (pond/plant) | | (pond/plant) | difference | weight (pond/plant) | difference |
| MESSENGER™ 2.2 oz/acre | 0.041 a* | 37.6 % | 0.505 a | 37.5 % | 0.546 | 37.5 % |
| Control (Grower standard) | 0.0298 b | , | 0.367 b | | 0.397 | |
| Level of statistically significant | P=0.119 | · | P=0.034 | | | P=0.033 |
| | | | | | | |

^{*} Same letter indicates no statistical difference between the two treatments at the defined level; different letter indicates a statistical difference between the two treatments at the defined level.

Table 8. Number of Bolls per 5 Plants at the Number 1 & 2 positions, and Total Number of Bolls from Whole Plants in Non-irrigated Cotton Following 26 days of drought.

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| Treatment | Avg. # bolls in the #1 & 2 position | Percent difference | Avg. # of total bolls per 5 plant | Percent difference |
|---------------------------------|---|-----------------------|--------------------------------------|--------------------|
| MESSENGER™ 2.2 OZ | 18.4 a | +46.0% | 21.4 a | +57.0% |
| Check | 12.6 b | | 13.6 b | - |
| Statistically significant level | P=0.032 | | P=0.01 | |

^{*} Same letter indicates no statistical difference between the two treatments at the defined level; different letter indicates a statistical difference between the two treatments at the defined level.

10 <u>Example.6</u> - Hypersensitive Response Elicitor-Treated Tomato is More Tolerant to Calcium Deficiency

Calcium is an important element for plant physiology and development. A deficiency in calcium can cause several plant diseases. For example, blossom-end rot is caused by a localized calcium deficiency in the distal end of the tomato fruit. Because calcium is not a highly mobile element, a deficiency can occur with a fluctuation in water supply. In the past, tomato growers experienced higher level of blossom-end rot during dry weather conditions when infrequent rains storms dumped a lot of water and then return to a hot and dry condition quickly. Lowering or raising the irrigation water table erratically during a dry and hot growing season can also increase the disease.

A field trial was designed to test if HR elicitor protein-treated tomato can be more tolerant to the calcium deficiency under a dry hot growing season.

MESSENGERTM, the formulated product containing 3% HR elicitor, was used for the trial. The application rate of the MESSENGERTM was 2.27 oz per care. The first spray of MESSENGERTM was carried out 7 days before the transplanting and then every 14-days after transplanting. MESSENGERTM-treated tomatoes were compared with a standard grower treatment not utilizing MESSENGERTM. Each treatment had 4 replicates.

The number of infected fruit was counted from a 100 square foot field. The rot typically begins with light tan water soaked lesions, which then enlarge, and then turn black. In a survey, about 20% of the fruits were infected. Severe end-rot

symptoms occurred in the standard treatment; however, an average of only 2.5 % of the fruit was infected in the MESSENGERTM-treated plants. The harvest data showed that MESSENGERTM-treated plants had 8% more marketable fruit (Table 9). The test results demonstrated that MESSENGERTM-treatment can reduce the stress resulting from calcium deficiency and increase plant resistance to blossom-end rot.

Table 9. Hypersensitive Response Elicitor Treatment Reduced Blossom-End Rot Infection, Increased Yield of Tomato Fruit

| Blosson | n-End Infe | ected Fruit* | | Tomato F | ruit Yield |
|---------|------------|-------------------|--------------------------|----------|-------------------------------------|
| Rep I | Rep II | Rep III | Rep IV | Bin/Acre | % Difference |
| 0 | 9 | 0 | 1 | 35 | 8 |
| 24 | 22 | 16 | 17 | 31.5 | • |
| | Rep I | Rep I Rep II 0 9 | Rep I Rep II Rep III | 0 9 0 1 | Rep I Rep III Rep IV Bin/Acre |

^{*}The data were collected from the fruits in 100 square foot plot

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and

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WHAT IS CLAIMED:

- A method of imparting stress resistance to plants comprising:
 applying a hypersensitive response elicitor protein or
 polypeptide in a non-infectious form to a plant or plant seed under conditions effective to impart stress resistance.
- A method according to claim 1, wherein the stress resistance is resistance to a stress selected from the group consisting of climated related stress, air
 pollution stress, chemical stress, and nutritional stress.
 - 3. A method according to claim 2, wherein the stress is chemical stress where the chemical is selected from the group consisting of insecticides, fungicides, herbicides, and heavy metals.
 - 4. A method according to claim 2, wherein the stress is climaterelated stress selected from the group consisting of drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light.
- 5. A method according to claim 2, wherein the stress is air pollution stress selected from the group consisting of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain.
- 6. A method according to claim 2, wherein the stress is nutritional stress where the nutritional stress is caused by fertilizer, micronutrients, or macronutrients.
- 7. A method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia, Pseudomonas, Xanthamonas, Phythophthera, or Clavibacter.

- 8. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Erwinia stewartii*.
- 9. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or *Pseudomonas solancearum*.
- 10. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Xanthamonas* species.
 - 11. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Phythophthera*.
- 12. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Clavibacter michiganesis* subsp. *sepedonicus*.
- 13. A method according to claim 1, wherein plants are treated during said applying.
 - 14. A method according to claim 1, wherein plant seeds are treated during said applying, said method-further comprising:
- planting the seeds treated with the hypersensitive response 25 elicitor protein or polypeptide in natural or artificial soil and propagating plants from seeds planted in soil.
- from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower,
 peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear,

melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

- 16. A method according to claim 1, wherein the plant is selected
 from the group consisting of Arabidopsis thaliana, Saintpaulia, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.
- 17. A method of imparting stress resistance to plants comprising:

 providing a transgenic plant or plant seed transformed with a

 10 DNA molecule which encodes for a hypersensitive response elicitor protein or
 polypeptide and

 growing the transgenic plant or plants produced from the
 transgenic plant seeds under conditions effective to impart stress resistance.
 - 18. A method according to claim 17, wherein a transgenic plant is provided.
 - 19. A method according to claim 17, wherein a transgenic plant seed is provided, said method further comprising:

 planting the transgenic seeds in natural or artificial soil and propagating plants from seeds planted in soil..
 - 20. A method according to claim 17, wherein the stress resistance is resistance to a stress selected from the group consisting of climated related stress, air pollution stress, chemical stress, and nutritional stress.
 - 21. A method according to claim 20, wherein the stress is chemical stress where the chemical is selected from the group consisting of insecticides, fungicides, herbicides, and heavy metals.

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PCT/US99/26039

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- 22. A method according to claim 20, wherein the stress is climaterelated stress selected from the group consisting of drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light.
- 5 23. A method according to claim 20, wherein the stress is air pollution stress selected from the group consisting of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain.
- 10 24. A method according to claim 20, wherein the stress is nutritional stress where the nutritional stress is caused by fertilizer, micronutrients, or macronutrients.
- 25. A method according to claim 20, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia, Pseudomonas, Xanthamonas, Phythophthera, or Clavibacter.
 - 26. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia amylovora, Erwinia carotovora, Erwinia chrysanthemi, and Erwinia stewartii.
 - 27. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or *Pseudomonas solancearum*.
 - 28. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Xanthamonas* species.
- from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic,

eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5 30. A method according to claim 20, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

SEQUENCE LISTING

<110> Eden Bioscience Corporation

<130> 21829/42

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<211> 1838

<212> PRT

<213> Erwinia amylovora

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Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly 35 40 45

- Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala 50 55 60
- Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
 65 70 75 80
- Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln 85 90 95
- Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala 100 105 110
- Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala 115 120 125
- Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met 130 135 140
- Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro 145 150 155 160
- Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln 165 170 175
- Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp 180 185 190
- Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile 195 200 205
- Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala 210 215 220
- Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Ser Glu Phe Gln Gln 225 230 235 240
- Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro 245 250 255
- Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys 260 265 270
- Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln 275 280 285

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Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val 290 295 300

- Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro 305 310 315
- Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys 325 330 335
- Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln 340 345 350
- His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr 355 360 365
- Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys 370 375 380
- Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys 385 390 395 400
- Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr 405 410 415
- Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
- Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
 435 440 445
- Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp 450 455 460
- Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp 465 470 475 480
- Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser 485 490 495
- Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser 500 505 510
- Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg 515 520 525
- His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His 530 535 540

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Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His 545 550 550 560

- Gly Lys Ser Glu Leu Glu Ala Gln S r Val Ala Ile Ser His Gly Arg
 565 570 575
- Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro 580 585 590
- Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
 595 600 605
- Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe 610 620
- His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg 625 630 635 640
- Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly 645 650 655
- Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His 660 665 670
- His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly 675 680 685
- Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr 690 695 700
- Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
 705 710 715 720
- Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu 725 730 735
- Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val 740 745 750
- Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
 755 760 765
- Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly 770 775 780
- Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe 785 790 795 800

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Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu 805 810 815

- Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His 820 825 830
- Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln 835 840 845
- Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Trp His Lys 850 855 860
- Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser 865 870 875 880
- His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln 885 890 895
- Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro 900 905 910
- Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met 915 920 925
- Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys 930 935 940
- Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val 945 950 955 960
- Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr 965 970 975
- Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His 980 985 990
- Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly 995 1000 1005
- Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro 1010 1015 1020
- Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His 1025 1030 1035 1040
- Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu 1045 1050 1055

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- Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly 1075 1080 1085
- Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu 1090 1095 1100
- Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu 1105 1110 1115 1120
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- Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val 1285 1290 1295
- Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
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- Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile 1330 1340
- Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys 1345 1350 1355 1360
- Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile 1365 1370 1375
- Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly 1380 1385 1390
- Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro 1395 1400 1405
- Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu 1410 1415 1420
- Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr 1425 1430 1435 1440
- Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn 1445 1450 1455
- Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser 1460 1465 1470
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- Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu 1555 1560 1565

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- His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu 1585 1590 1595 1600
- Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His 1605 1610 1615
- Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg 1620 1625 1630
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- Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser 1810 1815 1820

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Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His 45 40 35

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln 55 50

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met 80 70 65

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val 95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe 110 105 100

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu 125 120 115

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<213> Pseudomonas syringae

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Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala 50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val 65 70 75 80

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Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met 100 105 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro 145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe 165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile 180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly

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200

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Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser 210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser 225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp 245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln 275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala 290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala 305 310 315 320

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 65 70 75 80
- Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr 85 90 95
- Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln 100 105 110
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- Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly 145 150 155 160
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- Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
 195 200 205
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- Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp 225 230 235 240
- Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp 245 250 255

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Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr 260 265 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
275 280 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln 290 295 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala 305 310 315 320

Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp 325 330 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe 340 345 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln 355 360 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly 370 375 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr 385 390 395 400

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Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile

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| Ala | Ala 50 | Leu | Val | Gln | Lys | Ala 55 | Ala | Gln | Ser | Ala | Gly 60 | Gly | Asn | Thr | Gly |
|------------|------------|------------|------------|-----------|------------|------------|------------|------------|------------------|------------|------------|------------|------------|------------|------------|
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| - | | _ | 180 | | | | | 185 | | | | | 190 | Gly | |
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| _ | 210 | | | | | 215 | | | | | 220 | | | Gln | |
| 225 | _ | | | | 230 | • | | | | 235 | | | | Glu | 240 |
| | | | | 245 | | | | | 250 | | | | | Leu 255 | |
| | | | 260 | | | | | 265 | | | | | 270 | Asn | |
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| | 9 | D | 71 | 37. | 7 | C1 - | Dwc | C1 | Co- | 7.1 ~ | y cr | y cr | C1 - | Cor | 50~ |

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Leu Leu Ala Met

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INTERNATIONAL SEARCH REPORT

Inter onal Application No PCT/US 99/26039

| A CLASSI IPC 7 | FICATION F SUBJECT MATTER C12N15/82 C12N15/31 A01N63/0 |)2 | | | | | | |
|---|---|------------------------------------|-----------------------|--|--|--|--|--|
| According to International Patent Classification (IPC) or to both national classification and IPC | | | | | | | | |
| B. FIELDS | SEARCHED | | | | | | | |
| IPC 7 | Minimum documentation searched (classification system followed by classification symbols) | | | | | | | |
| | tion searched other than minimum documentation to the extent that s | | | | | | | |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used) | | | | | | | | |
| C. DOCUM | ENTS CONSIDERED TO BE RELEVANT | | | | | | | |
| Category * | Citation of document, with indication, where appropriate, of the rel | evant passages | Relevant to claim No. | | | | | |
| X | WO 98 32844 A (CORNELL RES FOUNDA 30 July 1998 (1998-07-30) claims | ATION INC) | 1-30 | | | | | |
| A | WO 96 39802 A (CORNELL RES FOUNDATION INC) 1-30 19 December 1996 (1996-12-19) claims | | | | | | | |
| A - | WO 98 24297 A (CORNELL RES FOUNDA 11 June 1998 (1998-06-11) claims | ATION INC) | 1–30 | | | | | |
| A | WO 98 37752 A (CORNELL RES FOUNDATION INC) 3 September 1998 (1998-09-03) | | | | | | | |
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| Further documents are listed in the continuation of box C. X Patent family members are listed in annex. | | | | | | | | |
| * Special categories of cited documents: "I" later document published after the international filing date | | | | | | | | |
| "A" document defining the general state of the art which is not considered to be of particular relevance. "A" document defining the general state of the art which is not cited to understand the principle or theory underlying the | | | | | | | | |
| "E" earlier document but published on or after the international "X" document of particular relevance; the claimed invention | | | | | | | | |
| "L" document which may throw doubts on priority claim(s) or involve an inventive step when the document is taken alone | | | | | | | | |
| which is cited to establish the publication date of another citation or other special reason (as specified) "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the | | | | | | | | |
| "O" document referring to an oral disclosure, use, exhibition or document is combined with one or more other such document other means document is combination being obvious to a person skilled | | | | | | | | |
| *P" document published prior to the international filing date but later than the priority date claimed in the art. *A" document member of the same patent family | | | | | | | | |
| Date of the | actual completion of the international search | Date of mailing of the internation | nal search report | | | | | |
| 22 May 2000 07/06/2000 | | | | | | | | |
| Name and m | nailing address of the ISA | Authorized officer | 10 44 541 | | | | | |
| | European Petent Office, P.B. 5618 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, | | G. W. C. | | | | | |
| | Fax: (+31-70) 340-3016 | Decorte, D | | | | | | |

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